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VOLUME 33 JULY 1955 NUMBER 4

AN APPARATUS FOR DETERMINING THE EFFECTS OF TEMPERATURE AND HUMIDITY ON GERMINATION OF FUNGOUS SPORES[1]

BY MARY P. HART[2] AND DONALD M. MACLEOD[2]

Abstract

An apparatus is described by which fungous spores can be uniformly distributed on microscope slides. A procedure is outlined whereby Petri dishes can be converted into airtight humidity chambers, the humidity of which can be readily checked. It was found that above 94% relative humidity maximum germination among *Beauveria bassiana* spores occurs at 28° C.

During an investigation to determine the optimal temperature and humidity ranges for the germination and growth of spores from a number of *Beauveria bassiana* strains, it became necessary to develop a method by which spores could be uniformly distributed on slides and incubated under accurately controlled conditions.

Various methods have been outlined for the application of spores to slides. For example, Yarwood (11) suggested that to ensure a more uniform distribution of the conidia on the test substrata, the slides, Petri dishes, leaves, or plants should be placed in the bottom of a large can and conidia dusted on them from above. Berwith (3) simply shook freshly collected conidia immediately on to dry glass slides in moist chambers maintained at different temperatures. Thirumalachar (10) placed a drop of sterile distilled water on a clean slide and added teleutospores; by spreading the drop with a glass rod, a thin smear was obtained on the slide. Snow (9) sowed the spores with a sterile platinum wire. Anderson (1) studied spore germination by placing two to four drops of a dilute conidial suspension of *Gibberella zeae* on a clean glass slide. At least 200 germinated and non-germinated conidia were counted on each slide and the percentage germination determined on the basis of these counts.

When these procedures were tested it was found that the spore distribution was uneven, numerous clusters occurring on the slides. As a rule clustered spores germinated more rapidly than did single spores and those on the periphery of a streak much more rapidly than the ones in the center.

[1] *Manuscript received March 18, 1955.*
Contribution No. 216, Forest Biology Division, Science Service, Department of Agriculture, Ottawa, Canada.

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In an attempt to obtain more uniform results, an apparatus was constructed similar to that described by Petty (5) for dusting insecticides. The spore dusting chamber (Fig. 1, *a*) consisted of a bottomless cellulose-acetate cylinder 12 in. in diameter and 16 in. high, with a 2-in. opening fitted with a rubber stopper (Fig. 1, *b*) in the center of the top. The spore container (Figs. 1(*d*) and 2) ,which dispersed the spores, was suspended in the dusting chamber at a fixed distance from the top. It consisted of a flat-bottomed glass vessel (Fig. 2, *a*) $2\frac{1}{2}$ in. high and $1\frac{3}{4}$ in. in diameter, with a tightly fitting rubber stopper (Fig. 2, *c*), through which passed four glass outlet-jets (Fig. 2, *d*) placed flush with the bottom of the stopper but extending $\frac{3}{4}$ in. through the top and drawn out to fine points 0.8 mm. in diameter. The openings of these tubes at the bottom of the stopper (Fig. 3) were covered with fine (50-mesh) wire gauze (Fig. 4). A fifth tube (Figs. 1(*c*) and 2(*b*)) of a slightly larger bore passed through the center of the rubber stopper and wire gauze and projected $\frac{3}{8}$ in. into the spore container. This tube had a spiral tip and was connected by a rubber tube to the air reservoir, from which air entered the spore container at a pressure of 5 lb. per sq. in., thus forcing the spores through the four outlet jets into the cylinder. In this way as many as 30 slides placed under the cylinder (Fig. 1) could be dusted with spores at one time.

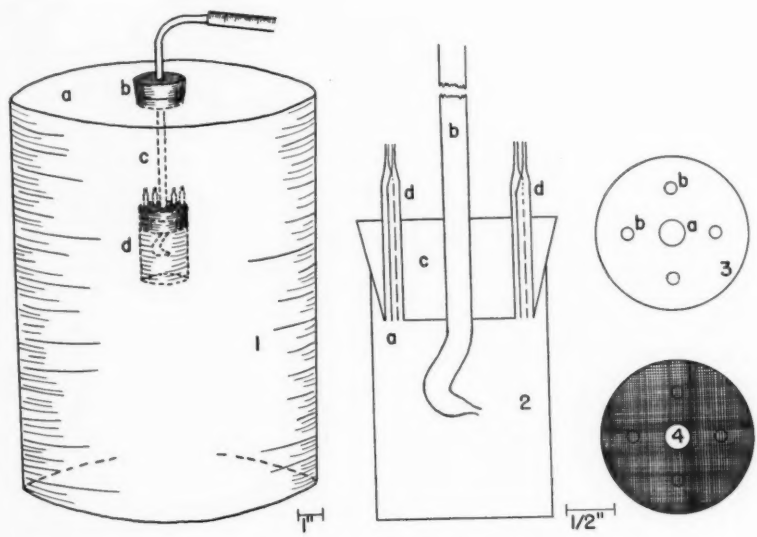

FIG. 1. Spore dusting apparatus: *a*, cellulose-acetate cylinder; *b*, rubber stopper; *c*, glass air tube; *d*, spore container. Fig. 2. Spore container enlarged: *a*, glass jar; *b*, glass air tube; *c*, rubber stopper; *d*, outlet jets. Fig. 3. Bottom of stopper in spore container without wire gauze: *a*, air tube; *b*, outlet jets. Fig. 4. Bottom of stopper in spore container covered with wire gauze.

Slides were prepared as follows. They were first washed with soap and water; then soaked in cleaning solution ($K_2Cr_2O_7$ 100 gm.; H_2SO_4 1000 cc.; H_2O 1000 cc.) for 24 hr., followed by immersion in a dilute solution of sodium hydroxide, and finally were rinsed several times in distilled water. All the glassware used was wrapped in paper and dry-sterilized for five hours at 160° C.

The test chambers were Petri dishes (150 mm. × 24 mm.) that had been made airtight by grinding both sections together with carborundum until a uniform seal was obtained. The top section was drilled in the center to take a stopper carrying the thermocouples used in checking the humidity. Normally the hole was closed by a solid stopper. Thus the conditions were upset as little as possible while measurements were being made.

The humidity in each test chamber was controlled with sulphuric acid to give relative humidities of 94 to 100%. One slide of each strain of fungus was placed in each chamber and supported above the acid solution on glass rods. Test chambers at the various relative humidities were then incubated at each of the test temperatures: 10°, 25°, 28°, 38°, and 44° C. During the experiments the humidity in the chambers was checked periodically by means of wet and dry thermocouples and a potentiometer (4, 6, 8).

In assessing germination, 36 areas on each slide were subdivided by means of a Howard ocular disk into 16 squares in which counts were taken daily.

The spores tested were from four morphologically distinct strains of *Beauveria bassiana*. Strains 1 and 2 were isolated from naturally infected hibernating larvae of the larch sawfly, *Pristiphora erichsonii* (Htg.), and strains 3 and 4 from larvae of the codling moth, *Carpocapsa pomonella* (L.).

Spores of each strain were removed from the surface of infected larvae of the host insect and injected into healthy larvae of the same species. The larvae were then kept in Petri dishes under laboratory conditions until they died. Upon mummification they were stored at about 0° C. for later use.

As fresh cultures of the fungus were required, mummified larvae were removed from cold storage, surface-sterilized in undiluted "Javex", and rinsed in three changes of sterile water for periods of one to two minutes each. They were then placed in Petri dishes on 20 cc. of agar (16 gm. Bacto-agar in 1000 cc. water) and incubated at 28° C. with continuous lighting for 50, 118, and 214 days. Spores were harvested by means of a sterile platinum loop for use in the germination tests.

The results obtained support earlier findings (2, 7). In general it was found that at relative humidity 94-100% maximum germination occurred at 28° C., was slightly less at 25°, and negligible at 10°, 38°, and 44°. At 94% relative humidity germination was very low, but as the humidity was increased above this value, there was a corresponding increase in germination at temperatures 25° and 28° C. Spores from 50- and 118-day-old cultures gave equally higher germination than did those from 214-day-old cultures.

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THE EFFECT OF EXTREME SHADE UPON LEAF FORM AND STRUCTURE IN *VICIA AMERICANA*[1]

By R. G. H. Cormack[2]

Abstract

A comparative study of light and shade leaflets of a common flowering plant (*Vicia americana*) reveals striking differences in leaflet form, size, thickness, and internal structure. The marked thinness of shade leaflets is due to the failure of the mesophyll cells to enlarge, and their peculiar folded form is attributed to the same cause.

Numerous studies have shown that leaves of the same plant species vary greatly in size and structure in relation to differences in light intensity. Some have dealt with differences in leaf structure among leaves from different locations on the same plant (2, 7), and others with effects of controlled environment on plants that normally produce either sun leaves, shade leaves, or both (3, 4, 5, 6).

The present study is an outgrowth of interest in sun and shade leaves in general (1) and in a recent paper by Wylie (8) in particular. Here he records variations in leaf structure among members of a colony of maidenhair ferns (*Adiantum pedatum* L.) growing in a rock cavern.

During the summer of 1952 attention was drawn to a flowering plant, namely American vetch (*Vicia americana* Muhl.), growing under conditions closely approximating those of the rock cavern described by Wylie. The unusual appearance of the leaves suggested that they might yield interesting material for a comparative anatomical study. This plant had taken root and was growing normally out-of-doors, but had also produced a single leafy shoot inside a dimly lighted shed. This shoot had grown up through the moist earth in a corner of the shed and at no time during its development had it been exposed to direct sunlight. By the middle of August the almost prostrate etiolated stem had grown to a length of some three feet and had produced a number of deformed flowering stalks and eight widely separated yellowish-green compound leaves. All the leaflets were exceedingly small and differed greatly from leaflets developed outside the shed in bright sunlight.

Materials and Methods

During the first week of August when all the leaves had reached their full maturity for the season, several typical leaflets were removed from both exposed and shaded shoots and killed in F.A.A. Some time later the leaflets were dehydrated in normal butyl alcohol, embedded in paraffin, and cut transversely at a thickness of 10μ. The sections were stained in Delafield's haematoxylin and safranin and then mounted in Canada balsam. At a later date epidermal peels were made from the same material.

[1] *Manuscript received February 21, 1955.*
Contribution from the Department of Botany, University of Alberta, Edmonton, Alberta.

[2] *Associate Professor of Botany.*

All leaflets from the shade leaves were extremely narrow and very sharply pointed at the apexes. They measured 12 to 14 mm. in length and 2 to 4 mm. at their greatest width. Closer inspection with a hand lens showed that they were all abnormal and that the abnormalities were associated with the complete or partial folding of the leaflets along their upper surfaces. Leaflets from the sun leaves, on the other hand, were uniformly flat and measured 32 to 35 mm. long and 11 to 12 mm. wide.

Results and Conclusions

Camera lucida drawings of transverse sections (Figs. 1–3) will serve to illustrate the main differences between light and shade leaflets. Casual comparison of Figs. 1*A–C* with Fig. 1*D* shows that the two kinds of leaflets differ markedly in size, thickness, and form. The most striking difference is the folding of shade leaflets (Figs. 1*A–C*) along their upper surfaces, as compared to the fully expanded condition of the sun leaflet (Fig. 1*D*). In Fig. 1*A* the leaflet is completely folded and thus appears to be double, while Figs. *B* and *C* show varying degrees of folding. The averages of measurements of the thickness of sun and shade leaflets are 137μ and 76μ respectively.

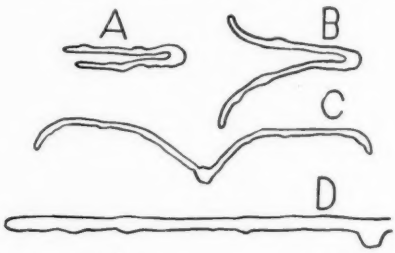

FIG. 1. (×12) *A–C*, complete cross sections of shaded leaflets, showing small size and folded shape. *D*, cross section of one half of a typical sun leaflet.

Comparison of Fig. 2*a* with Fig. 2*b* reveals further differences and may throw some light on the cause of these differences. In both sun and shade leaflets the mesophyll is seen to consist of four layers of cells. In the former it is clearly differentiated into palisade and spongy mesophyll while in the latter the palisade layer is scarcely discernible. The palisade cells have an average length of 58μ in sun leaflets and 19μ in shade leaflets. Not only are the palisade cells longer in the sun leaflets but they are loosely arranged, completely vacuolated, and supplied with numerous large chloroplastids. On the other hand, the palisade cells of the shade leaflets are closely packed together, partly vacuolated, and most of them contain small oval-shaped chloroplastids. The spongy mesophyll cells also differ in the same respects.

Not only are the chloroplastids large in sun leaflets (Fig. 3*a*) but they are clearly discernible when viewed by either transmitted light or by phase-contrast illumination. On the other hand, those of shade leaflets (Fig. 3*b*) are scarcely distinguishable as definite chloroplastids in transmitted light but

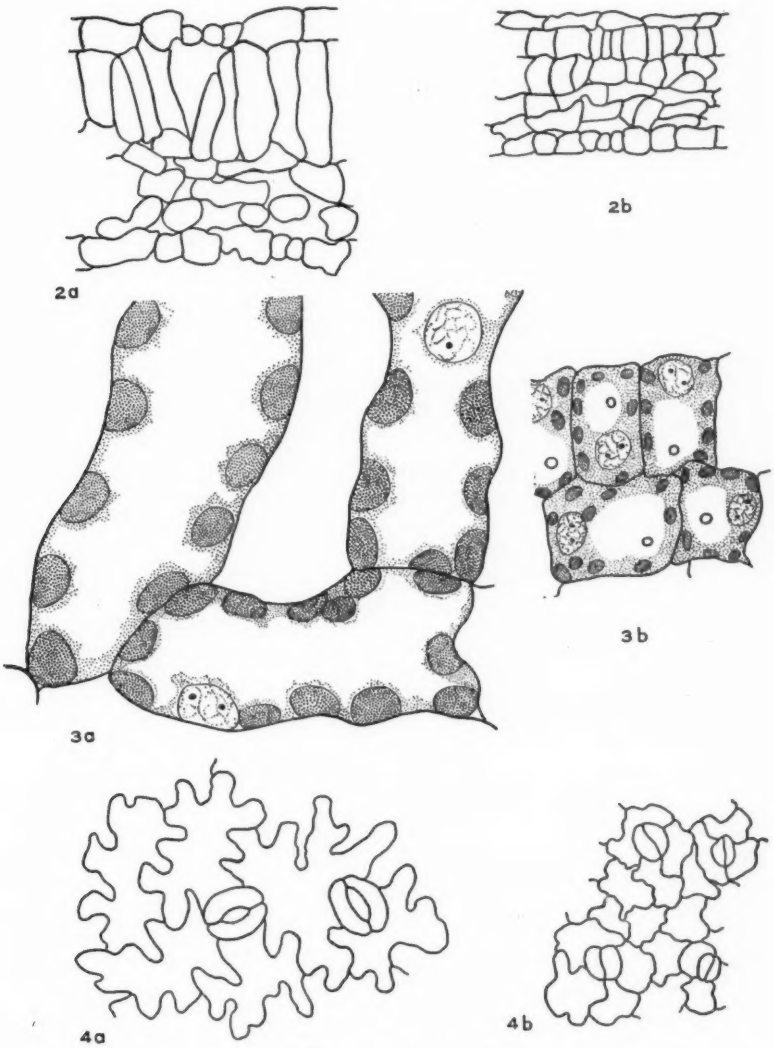

FIG. 2. Cross sections of sun and shade leaflets. *a*, from shoot in direct sunlight; note well-differentiated palisade layer. *b*, from deep shade; number of cell rows the same as in *a*, but no distinct palisade. (×300)

FIG. 3. Mesophyll cells from leaflets of Fig. 2. *a*, palisade (upper row) and spongy parenchyma cells of sun leaflet. *b*, corresponding cells of shade leaflet. For further description see text. (×1000)

FIG. 4. Lower epidermis of sun and shade leaflets, *a* and *b* respectively, showing differences in cell size and condition of the guard cells. (×300)

are clearly defined as such under phase-contrast illumination at exactly the same magnification. This difference in discernibleness of the chloroplastids suggests some difference in the state of the cytoplasm in which they are embedded. In the mesophyll cells of shade leaflets the cytoplasm appears to be very dense and sharply marked off from the small spherical vacuoles. In those of sun leaflets the cytoplasm appears dispersed and there is no distinct boundary between it and the large central vacuole. In addition to the nucleus and chloroplastids there is present a single small translucent spherical body in the vacuole of mesophyll cells of shade leaflets (Fig. 3*b*). Under phase-contrast illumination this small but definite body does not have the same consistency as either nucleus or chloroplastids. This peculiar body does not occur in the mesophyll cells of sun leaflets (Fig. 3*a*). Its true nature remains a matter of conjecture.

The areas of shade leaflets are considerably less than those of sun leaflets. In comparing total leaflet areas of fully developed leaflets, shade leaflets have only 20–25% of the area of sun leaflets. As evident from a comparison of lower epidermal peels (Figs. 4*a–b*) there is a corresponding decrease in surface areas of individual epidermal cells. It is also obvious that the stomata which occur on both surfaces are much more numerous per unit area in shade leaflets than in sun leaflets. Another difference of considerable interest in this connection is the condition of the guard cells. In Fig. 4*a* the guard cells of sun leaflets are seen to be large, fully vacuolated, and widely open, while those of the shade leaflets in Fig. 4*b* are smaller, and tightly closed. With phase-contrast illumination chloroplastids are clearly visible in the guard cells of sun leaflets and barely discernible in those of shade leaflets. The cuticle is relatively thin in both leaflets but appreciably thinner in the shade leaflets than in the sun leaflets.

Another notable feature that bears out the undifferentiated condition of the shade leaflets is the development of the vascular tissue. Weakly lignified xylem elements are few in number in the main vein and absent completely in the laminae of shade leaflets. In the laminae, veins are distinguishable as groups of small centrally placed cells containing nuclei and considerable protoplasm. The same holds true for elements of the phloem in shade leaflets. In sharp contrast both xylem and phloem are well developed in sun leaflets. Not only are the xylem elements numerous but their walls are thick and strongly lignified.

The present study is a comparison of changes produced in leaflets of a flowering plant under conditions of extreme shade similar to those described by Wylie for maidenhair ferns growing in and near the entrance of a rock cavern (8).

In Wylie's study, the ferns in deep shade at the back of the cavern produced the smallest and thinnest leaves, while those growing a few feet outside the cave entrance produced the largest and thickest leaves. Deeply shaded pinnules possessed no mesophyll, while exposed pinnules had always two and sometimes three cell rows of that tissue. His observation that the deeply

shaded pinnules were thinner than exposed pinnules agrees with observations of extreme shade leaflets of the American vetch plant described in the present study. However, in Wylie's ferns the extreme thinness of deeply shaded pinnules was due to the complete absence of mesophyll, while in the vetch it was due entirely to the failure of the mesophyll cells to enlarge, the number of cell rows remaining constant. Moreover the peculiar folded condition of the shade leaflets could be explained by the lack of elongation of the mesophyll cells.

The present study was limited to a description of leaflets developed naturally under different conditions of light intensity. An experiment designed to throw some light on the fundamental problems of cell elongation and differentiation responsible for the structural differences described in the present paper is now in progress.

Acknowledgments

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A FLOTATION METHOD FOR OBTAINING SPORES OF *HELMINTHOSPORIUM SATIVUM* FROM SOIL[1]

By R. J. Ledingham[2] and S. H. F. Chinn[3]

Abstract

This paper describes a flotation method by means of which spores of *Helminthosporium sativum* may be recovered from soil. Soil mixed with a small amount of mineral oil is shaken up in water. The emulsion which collects on the surface of the water contains most of the spores originally in the soil. Approximate spore numbers are determined by microscopic examination of aliquots of the emulsion. The technique is also well adapted for obtaining spores from soil for viability studies. It may be useful in comparing the effects of cultural and chemical treatments of soils on spore populations under field conditions.

Introduction

Fungi in soil have been recognized chiefly by direct examination or by isolation and cultural methods. The numerous innovations in connection with direct examination methods have been reviewed by Blair (2). Isolation and cultural techniques have received considerable attention and include methods such as isolation from suitable hosts, isolation from selective media placed in the soil, and direct isolation from soil using various kinds of media. The method outlined in this report utilizes surface tension phenomena and appears well adapted for recovering large-sized spores from soil. Preliminary work on the technique has already been reported (4).

Many soil samples have been examined for the presence of spores of *Helminthosporium sativum* P.K. and B. Data presented below record the number of spores found in a few samples of soil from cultivated fields in Saskatchewan.

The Flotation Method

Soil for examination is screened to remove the larger bits of trash and plant debris. Water is added to bring the soil to a moisture level of 10% by weight. A 10 gm. sample is weighed out and mixed with 5 ml. of mineral oil (Nujol) in a watch glass. The mixture is next transferred to a test tube, 25 by 250 mm. size, and 50 ml. of tap water is added. The tube is agitated vigorously for four or five minutes, then placed in a vertical position. In about half an hour, depending on soil type, most of the soil has settled and an emulsion has collected at the surface (Fig. 1). Approximately six milliliters of emulsion collects when 5 ml. of oil are used. When necessary, excess soil particles in the emulsion are settled out by addition of a small amount of sodium chloride or other electrolyte. Aliquots of the emulsion are transferred to microscope

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Joint contribution from the Botany and Plant Pathology Division (Contribution No. 1438) and from the Bacteriology Division (Contribution No. 384) Science Service, Canada Department of Agriculture, Ottawa, Ontario.
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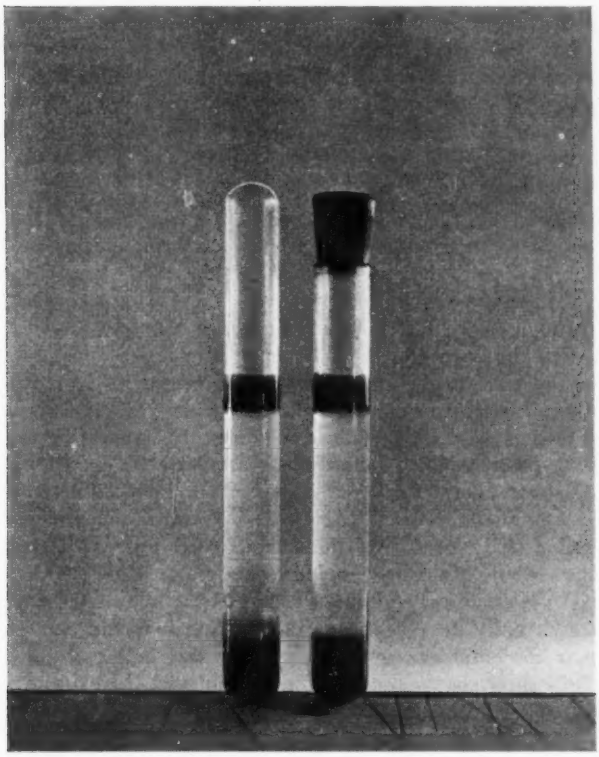

FIG. 1. The flotation method showing the settled or extracted soil at the bottom of the tubes, the water phase next to the soil, and the emulsion above the water. Right, regular setup. Left, vial fastened to the end of tube in order to collect the extracted soil for plating.

slides for examination. Droplets of oil and small air bubbles in the emulsion on the slide are broken down by agitation with a needle and the mixture is spread in a film. A wide-field binocular with a magnification of 72× is used for observing and counting the spores. The term 'flotation count' is applied to determinations of spore numbers by this method.

A sample of the emulsion is obtained with a pipette which delivers drops of 1/50 ml. volume. The pipettes are drawn out from glass tubing. The spores in at least ten drops are counted, and the approximate total number of spores in the emulsion, and hence spore numbers per gram of soil, are computed.

Efficiency of the Flotation Method in Recovering Spores from Soil

In order to determine the effectiveness of this technique in removing spores from soil, the dilution plate method was employed to find the percentages of

spores held in the emulsion layer, in the water layer, and in the soil layer. Soils of various types were steam sterilized in a moist condition then dried until the moisture content was about ten per cent. Conidia were collected by shaking some dry, sterile sand in Petri dishes containing a two- to three-month-old culture of *H. sativum*. Portions of this heavily infested sand were added to the sterile soil in large flasks. Uniform distribution was achieved by rotating the flasks.

Ten-gram samples of the inoculated soils were weighed out and used directly in a dilution plate series to determine the spore population of each soil. Other 10 gm. portions were mixed with oil and placed in test tubes, and water was added in the amount mentioned above. Instead of a stopper, a vial of the same diameter as the test tube, and just long enough to hold all the soil, was fastened to the open end of each test tube with cellulose tape (Fig. 1, left). After the tubes were shaken they were placed in a vertical position with the vial end down so that the soil might collect at the bottom. When the soil had settled the vials were removed carefully by severing the tape and quickly sliding them off so as to avoid contact with the emulsion. The soil from the vials was plated to determine spore numbers. The emulsion also was plated in some of the tests. Removal of the emulsion was achieved by pipetting, followed by swabbing the sides of each tube with sterile cotton swabs to recover oil and spores clinging to the glass. In several cases water from between the emulsion and the settled soil was pipetted off and plated. It contained virtually no spores and this finding indicated that the spores were in either the emulsion or the soil, or divided between the two. The data in Table I show spore counts of the original soil, the extracted soil, and the emulsion, of several soil types.

TABLE I

PLATE COUNTS SHOWING SPORE NUMBERS IN THE ORIGINAL SOILS, THE EXTRACTED SOILS, AND THE EMULSIONS, AND ALSO THE PERCENTAGE OF SPORES STILL REMAINING IN THE SOILS AFTER EXTRACTION

Soil type	Original soil	Extracted soil	Emulsion	$\frac{\text{Extracted soil}}{\text{Original soil}} \times 100$
				%
Heavy clay	1950	240	—	12
Silty loam	2200	100	—	5
Clay loam	900	150	—	17
Sandy loam	2000	130	—	7
Gray bush	1170	150	950	13
Medium loam	1370	320	1000	24
Medium loam	1390	280	1260	20
Medium loam	1210	250	1350	21
Mean	—	—	—	15

The data in Table I show that relatively few spores remained in the various soils after the flotation treatment. Actually the percentages ranged from

5 to 24% with an average of about 15%. The remaining 85% presumably was held in the emulsion. The four samples cited in Table I, which is concerned with plated emulsions, indicate that this was the case. In two instances the total of spore numbers in the emulsion and the extracted soil was slightly higher than in the original soil; however, these are variations which may be expected. The main point of interest brought out in Table I is that the flotation method is an effective means of recovering, in the emulsion phase, a high percentage of spores of *H. sativum* from soil.

Comparison of Flotation Count and Plate Count

Most of the sterilized and subsequently inoculated soils of the preceding section not only were plated to determine spore numbers but were used in flotation count determinations. The data presented in Table II show the relation between the flotation counts and the spore numbers of the soils as determined by plating.

TABLE II

COMPARISON OF SPORE NUMBERS AS DETERMINED BY THE FLOTATION COUNT AND BY PLATING

Soil type	Flotation count	Plate count	$\frac{\text{Flotation count}}{\text{Plate count}} \times 100$
			%
Clay loam	600	900	66
Silty loam	1600	2200	73
Sandy loam	1300	2000	65
Sandy loam	800	820	97
Sandy loam	600	780	77
Sandy loam	450	635	71
Gray bush	700	1170	59
Medium loam	1200	1370	87
Mean	—	—	74

The flotation count of spores in the soils varied somewhat from the numbers determined by plating. These variations are due to at least two causes. First, as shown in Table I, there is variability in the percentage of spores recovered from different soils and held in the emulsions. Secondly, depending on thoroughness of sampling, care in examining the samples and so forth, inaccuracies occur in estimating spore numbers in the emulsion. The flotation count, as indicated in Table II, generally is lower than the plate count. Considering the eight samples for which data are presented, the flotation count averaged 74% of the plate count, with a variation in the different samples from 59% to 95%.

Differences in results obtained by the flotation technique do not seem to be related to difference in soil type. The flotation counts deviated as much from the plate count in samples of a single soil type as between samples of different soil types.

An Application of the Flotation Method

Twenty soil samples collected in April 1954 from fields around Saskatoon were examined by the flotation method for numbers of spores of *H. sativum*. The results are presented in Table III. It is not intended at this time to try to relate these spore populations to cultural or cropping practices.

TABLE III

NUMBERS OF *H. sativum* SPORES IN SOIL FROM 20 CULTIVATED FIELDS NEAR SASKATOON

Spores per gram of soil	No. of fields
Less than 25	1
Between 25 and 50	9
Between 50 and 100	4
Between 100 and 150	4
More than 150	2

The data in Table III indicate the incidence of spores of *H. sativum* in this district. It is noteworthy that none of the soils examined, including several garden soils, were entirely free of spores. It is known that spores are produced in abundance on the crowns of infected wheat plants or other susceptible hosts. Their presence in garden soils suggests that they may have been carried there by wind. In fields cropped to susceptible hosts such as wheat or barley, the spores produced on the plant parts are disseminated through the soil during cultivation.

Discussion

The prevalence of infections caused by *H. sativum* on wheat plants grown in prairie soils indicates that this fungus is widespread in such soils, either in the spore or mycelial form. The dilution plate method apparently is not well suited to demonstrate the presence of the fungus. Henry (3) was unsuccessful in attempts to isolate *H. sativum* from soil in Minnesota and found it only rarely in Alberta soils. Bisby *et al.* (1), after completing a series of isolations, refer to its "sparse occurrence" in Manitoba soils.

The main problem in isolation of *H. sativum* from soil appears to be its relative scarcity in comparison with other fungi. Bisby *et al.* (1) obtained seven colonies of *H. sativum* out of a total of 7000 that developed in an isolation series; the ratio was thus 1 : 1000. Based on the total fungus count for that particular soil they calculated the *H. sativum* content to be about 90 spores or bits of mycelium per gram of soil. The results in Table III show that this number of spores of *H. sativum* is not unusual in soil around Saskatoon.

The proportions of oil, soil, and water as given for the flotation technique are not necessarily the only ones that will give good spore recovery. Twenty

grams of soil mixed with 5 cc. of oil may be preferable, particularly in soils with relatively few spores.

In the computation of total spore numbers it should be kept in mind that by the flotation method only 80 to 90% of the spores are recovered from the soil and a further 15 to 20% are overlooked in examining the emulsion; hence adjustment should be made to this extent. The adjustment is less important in comparative studies of soils that have undergone various chemical treatments or have been subjected to different cultural and cropping practices. In such cases the determination of relative spore numbers suffices. In any event determinations of spore numbers will be only approximate, their degree of reliability depending on factors such as thoroughness of sampling of the emulsion and accuracy of the various measurements involved.

In the dilution plate counts it might be expected that mycelial fragments of *H. sativum* would be responsible for a considerable number of colonies, and would thus render invalid a comparison between the flotation count and the plate count. The writers found, however, that the inoculum obtained from two- to three-month-old cultures of *H. sativum* maintained on potato-dextrose agar at room temperature contained comparatively small amounts of mycelial fragments. Moreover, only a low percentage of the fragments were capable of resuming growth, even under favorable conditions. It appears then that the plate counts were influenced only slightly by mycelial fragments so that the plate count as well as the flotation count was a measure of spore numbers. Germination studies showed that spores from the cultures used in inoculating the soil were almost all viable, so that a comparison of the flotation count and the plate count seems to be justified.

Success in determining spore numbers in soil by the flotation method may be expected only with the larger-spored species of fungi. On the other hand, smaller spores such as those of *Fusarium* spp. may be recovered from soil for germination and longevity studies by this method.

The writers found the technique useful in obtaining spores from field soil for germination and longevity studies. Several fungi other than *H. sativum* have been handled in the same way.

Acknowledgments

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SEDIMENTARY CHLOROPHYLL DETERMINATION AS A PALEOBOTANICAL METHOD[1]

By J. R. Vallentyne[2]

Abstract

Chlorophyll degradation products which can be extracted from sediments with 90% acetone and which show maximal light absorption in the red end of the spectrum at a wave length of 667 mμ (90% acetone as the solvent) are here collectively defined as sedimentary chlorophyll. A quantitative method is described for the determination of sedimentary chlorophyll. Results from six different sediment profiles are presented. The nature of sedimentary chlorophyll is unknown, except that it consists of at least three chlorophyll degradation products. Sedimentary chlorophyll has been found in samples aged up to 11,000 years. In any one profile the fluctuations in sedimentary chlorophyll content may be used to infer changes in the abundance of chlorophyll-bearing plant tissues, but only with the greatest caution.

Introduction

Both marine and fresh-water phytoplankton have been extensively studied by ecologists because of the importance of these communities in synthesizing organic matter. Aside from microscopic counts, the first practical method of measuring phytoplankton populations separately from those of the zooplankton was the method of pigment analysis developed by H. W. Harvey (7). The basis of Harvey's method was extraction of chlorophylls and carotenoids from plankton samples with acetone, and determination of the color density by comparing unknowns to a series of standards. This ingeniously simple method has been developed and extended by several workers, notably Riley (15), Gardiner (6), Tucker (17), and Richards and Thompson (14).

During a study of Connecticut lake sediments the author found that acetone extracts of sediments were almost invariably colored green. The absorption spectra of the crude extracts were similar, but not identical, to those of the known chlorophylls. This immediately suggested the possibility of using the chlorophyll degradation products of sediments as biochemical fossils. A preliminary account of the results obtained from this work has been published (18).

Chlorophyll-like material had previously been found in subaquatic sediments by Lubimenko and Rauser-Cernooussova (11), Baudisch and von Euler (1), Jastrebova (9), Klenova and Jastrebova (10), Fox, Updegraff, and Novelli (5), and Phinney (13). Attempts to recover chlorophylls *a* and *b* from sediments have failed (5, 13). The green lipoids of sediments are

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Joint contribution from the Department of Biology, Queen's University, Kingston, Ontario, and the Osborn Zoological Laboratory, Yale University, New Haven, Connecticut. Part of this work was presented in a thesis submitted to the Graduate School of Yale University in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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evidently all chlorophyll degradation products. No one had determined the amounts of chlorophyll degradation products at more than a few selected levels of a sediment profile. Our purpose was to determine the amounts of chlorophyll degradation products at successive levels in a column of lake sediment, in the hope that the concentrations might be used as indices of the abundance of past plant-populations. The need for such an approach is obvious when one considers the great susceptibility of plant tissues to morphological decay. The importance of a biochemical paleobotany is that it offers a new means of measuring past plant-populations, by a method which is to some extent independent of the preservation of biological form. The chemical structures however may be just as susceptible to decay as the morphological, if not more so. Only by the combined attack of several different approaches will it be possible to unravel the complex changes in plant associations which have occurred in past time.

Methods and Materials

The method outlined below for sediments is similar to the procedures used in the determination of phytoplankton chlorophyll. About two grams wet weight of sediment is homogenized on a glass plate with a spatula. Half the sample is removed, weighed to the nearest milligram, and placed in a test tube 25 mm. in diameter and 150 mm. high. The extraction solvent is prepared before hand: 90% acetone containing 0.5% of redistilled dimethylaniline. About 30 ml. of the extraction solvent is thoroughly mixed with the mud (using a glass rod) and the tube is left to stand overnight with occasional shaking. The other half of the homogenized mud sample is weighed to the nearest milligram and then dried at 100° C. for 20 hr. in a drying oven. At the end of that time the sample is reweighed and the loss in weight calculated to give the water content. In this way the sedimentary chlorophyll results can be referred to a unit dry weight without actually drying the sample used for the sedimentary chlorophyll analysis. The dried mud sample may be used for the determination of ignitable matter (which gives an approximate measure of the organic content). When sedimentary chlorophyll values are referred to a unit weight of ignitable matter, the results become independent of the rate of mineral sedimentation.

After the mud has stood overnight in the solvent, the supernatant is poured off and filtered through Whatman No. 50 filter paper. The residue is repeatedly extracted until all the color has been removed. The pigments tend to creep up the paper during filtration and must be washed down with small portions of solvent. It is convenient to collect the filtrate directly in a 100-ml. graduated cylinder. This gives an immediate measure of the filtrate volume. After the volume has been recorded, the filtrate is thoroughly

mixed. An aliquot is added to a Beckman absorption cell (10-mm. light path) and the solvent is used as a blank in a matched cell. Color density is determined with a Beckman Model DU quartz spectrophotometer at a wave length of 667 mμ[3] using a slit width of about 0.05 millimeters. The reading is made on the density scale, rather than on the per cent transmission scale. Owing to improper calibration, the wave length of maximum density may vary with different spectrophotometers.

Since the structures of the chlorophyll degradation products are unknown the results cannot be expressed as weight per unit weight of mud. Results are expressed in terms of arbitrary units. The complex of chlorophyll degradation products will hereafter be called sedimentary chlorophyll, and the concentration units called sedimentary chlorophyll units. A sedimentary chlorophyll unit, as here defined, is a function of the density reading given by a Beckman DU quartz spectrophotometer *at the wave length of maximum absorption* in the red end of the spectrum using absorption cells with a 10-mm. light path and a blank of the pure extraction solvent (90% acetone with 0.5% dimethylaniline). Under these conditions, one sedimentary chlorophyll unit is defined as giving a density reading of 0.100 when dissolved in 10 ml. of solvent. An example calculation is given below:

The extract (48 ml. in volume) from 0.161 gm. dry weight of sediment had a density ready of 0.131.

$$\text{Sedimentary chlorophyll} = \frac{\text{Density reading} \times \text{filtrate vol. (ml.)}}{\text{Dry wt. of mud extracted (gm.)}} = \frac{0.131 \times 48.0}{0.161} = 39.0 \text{ units per gm. dry weight.}$$

Table I shows the analytical results for six aliquots taken from a homogeneous fine-grained sediment sample from Lower Linsley Pond, Connecticut. The results are reproducible to within 2%. Results from coarse-grained sediments are more variable, but are reproducible to within 5–10% at the worst. The variability is reduced if coarse-grained samples are ground in a mortar previous to extraction. The solutions obey Beer's Law over the density range 0.030–0.600.

In most of our early analyses for sedimentary chlorophyll (including all results contained here) dimethylaniline was not added to the 90% acetone. Tests on a homogeneous sediment sample show that when dimethylaniline is added to 90% acetone the sedimentary chlorophyll values are approximately 10% higher than for 90% acetone alone. If magnesium carbonate is added to 90% acetone during extraction, the sedimentary chlorophyll values may be as

[3] *We have recently found one lake where the maximum falls from 667 mμ for surface sediment to 663 mμ for sediment 40 cm. below the surface.*

TABLE I

SIX DETERMINATIONS OF WATER CONTENT AND SEDIMENTARY CHLOROPHYLL ON ALIQUOTS OF A HOMOGENEOUS SEDIMENT SAMPLE TAKEN FROM LOWER LINSLEY POND, CONNECTICUT

% H_2O	Dry wt. extracted, gm.	Sedimentary chlorophyll, units/gm. dry wt.
86.0	0.108	28.3
86.0	0.090	27.9
85.9	0.091	27.7
85.9	0.033	28.2
85.7	0.101	27.8
85.8	0.026	27.3
Av. = 85.8		Av. = 27.9
S.D. = ±0.1		S.D. = ±0.4

NOTE: *S.D. = Standard deviation.*

much as 20% lower than for 90% acetone alone. Since dimethylaniline is a liquid base (no adsorption of pigments), its use as an antacid reducing agent is preferred, but it should be used only if one has access to a fume hood.

The effect of postcollection changes on the sedimentary chlorophyll content of mud has been tested in several ways. One of the more serious effects could be oxidation. When sediment profiles are taken from a lake bottom the mud is initially reduced, often smelling of hydrogen sulphide. Processes of oxidation during laboratory storage could conceivably destroy some sedimentary chlorophyll. To test this possibility a reduced mud sample was thoroughly oxidized for two days by continuously bubbling air through a mechanically stirred suspension. The sedimentary chlorophyll content was not changed by this drastic procedure. It has also been found that samples of mud stored at room temperature in the laboratory show no change in sedimentary chlorophyll content for periods of at least up to a year.

The drying of sediment samples at room temperature does not seriously affect the sedimentary chlorophyll, providing that the dried samples are moistened for several hours before extraction. Low values are obtained if the samples are not moistened before extraction, presumably because extraction is difficult under these conditions (16).

If a concentrated acetone extract of a lake sediment is streaked along the base of a sheet of Whatman No. 1 filter paper and the chromatogram developed with petroleum ether, three separate chlorophyll degradation products can be

seen. Their positions on the paper are shown in Fig. 1, with the paper oriented in its natural position for an ascending chromatogram. The absorption maxima for the upper two chlorophyll degradation products lie at 667 mμ, while that for the lowermost is at 667 mμ in all lakes studied except one. Mr. S. R. Brown (personal communication) found that the lowermost chlorophyll degradation product in sediments from Little Round Lake (near Maberly, Ontario) had a maximum at 659 mμ. This would suggest that two or more compounds occupy that position. In all cases the maxima refer to those found in 90% acetone solutions.

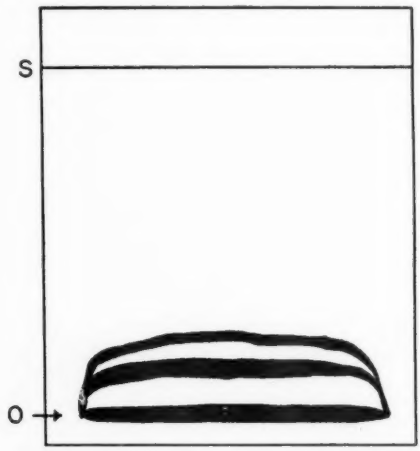

FIG. 1. A diagram of the positions of three chlorophyll degradation products on an ascending paper chromatogram developed with petroleum ether. The R_f values vary directly with concentration, hence the inverted U-shape. Solvent front (*S*) and origin (*O*) as indicated.

Results

Fig. 2 shows the general similarity of absorption curves for crude 90% acetone extracts of plankton and sediment from Lake Opinicon, Ontario. The plankton sample (net plankton) was collected in August during the height of a *Lyngbya* bloom. The sediment sample was collected with the author's piston sampler (19) one meter below the surface sediment. The volumes of the two extracts were adjusted so that the density readings at 666 mμ were identical. At all wave lengths except 666 mμ the light absorbed by the sediment extract was greater than that absorbed by the plankton extract. The density-differences between the two curves are plotted as a separate curve in Fig. 2. The peak absorption of the density-difference curve was located at 450 mμ, i.e. very close to the absorption maxima exhibited by acetone solutions of algal carotenoids (14). Carotenoids show absorption below 400 mμ, and since the density difference curve does not, it is clear that

other visible light-absorbing compounds may be present. Fig. 2 is presented to show the spectral similarity of plankton and sediment extracts. The single plankton sample cannot be compared directly to the sediment because the former is an isolated sample representative of one time of the year, whereas the latter gives an average for the sediments deposited over a period of several years.

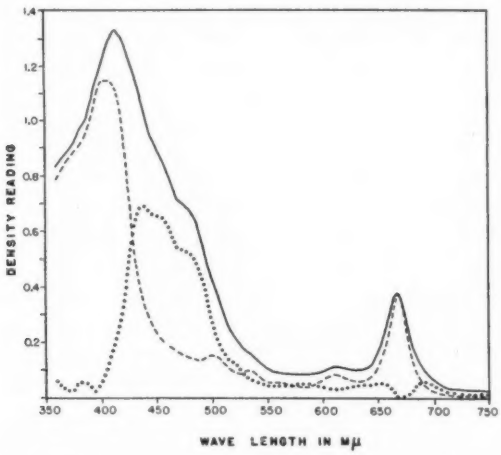

FIG. 2. Light absorption curves for 90% acetone extracts of plankton (– – – –) and sediment (————) from Lake Opinicon, Ontario, together with the curve showing the density differences (. . . .).

Sedimentary chlorophyll data are shown in Fig. 3 for sediment profiles taken from six different lakes. Curves for the percentage of ignitable matter are given for five of the lakes. The low chlorophyll and ignitable matter values at the bottom of each profile, except that from Muskee Lake (Fig. 3C), simply reflect the silt-clay nature of the lowermost samples. The basal part of the Muskee Lake profile was organic, but was diluted with sand and pebble particles.

The results from Bethany Bog (Fig. 3A) and Upper Linsley Pond (Fig. 3B) are of interest because these profiles have been analyzed for pollen, and hence a relative chronology is available. The 12 meter level of the Bethany Bog profile and the 11 meter level of the Upper Linsley Pond profile lie in the *B* pollen zone of Deevey's (2) chronology. The top of the *B* pollen zone in Upper Linsley Pond has been dated at 8323 ± 400 years by the radiocarbon method (3). The presence of sedimentary chlorophyll at these levels gives some indication of the length of life of the chlorophyll degradation products in sediments. Sedimentary chlorophyll has also been isolated from sediments 11,000 years old in Lower Linsley Pond.

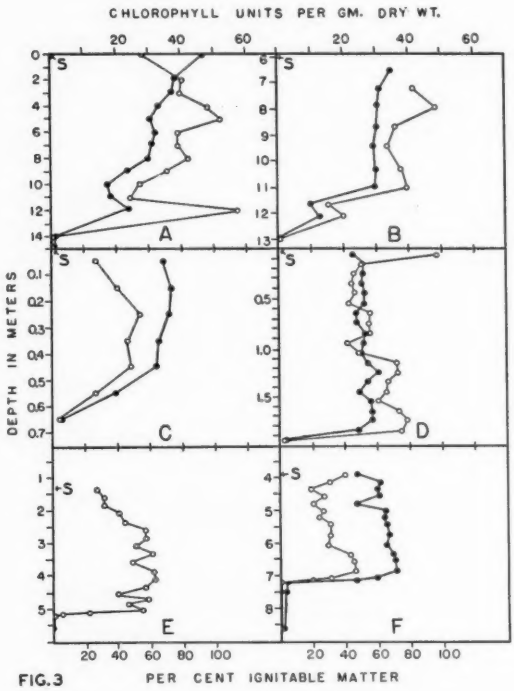

FIG. 3

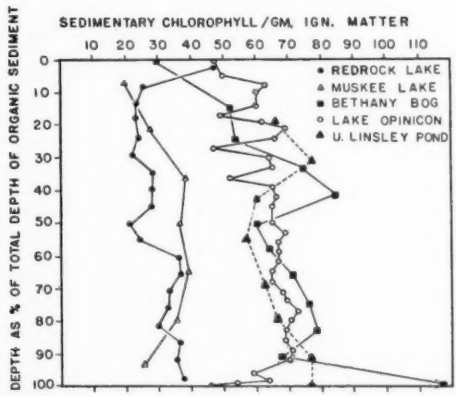

FIG. 4

FIG. 3. Curves for sedimentary chlorophyll (open circles) and ignitable matter (solid circles) in lake sediment profiles. A, Bethany Bog, Connecticut; B, Upper Linsley Pond, Connecticut; C, Muskee Lake, Colorado; D, Redrock Lake, Colorado; E, Lake Irene, Quebec; F, Lake Opinicon, Ontario. For A, B, E, and F the depths refer to the distance below the water surface. The depth of the mud surface is shown by "*s*". For C and D the depths refer to the distance below the mud surface.

FIG. 4. Curves for sedimentary chlorophyll (units per gm. ignitable matter) in cores taken from five lakes. See text for explanation of depth scale.

The profiles from Muskee and Redrock Lakes (Fig. 3, C and D), Colorado, were collected by R. W. Pennak. They are currently being analyzed for other fossil constituents. The profile from Lake Irene (Fig. 3E), Quebec, was collected by H. Ignatius. The samples provided were too small for the determination of ignitable matter. The abrupt rise in sedimentary chlorophyll values at 5.2 meters was largely due to a decreasing proportion of silt and clay. Fig. 3E shows the sedimentary chlorophyll and ignitable matter curves for a shallow water profile (3.9 meters depth) taken from Lake Opinicon, Ontario. At 7.18 meters depth the sediment abruptly changed from a silt-clay below to a gyttja above. The boundary was exceedingly sharp, and the silt-clay devoid of sedimentary chlorophyll. A profile taken in deeper water (6.5 meters) in the same lake showed roughly the same trends in the sedimentary chlorophyll curve, but the amount of chlorophyll in a unit weight of ignitable matter was nearly twice that found in the shallow water profile. It is clear that a single profile from one lake does not give an adequate picture of the sedimentary chlorophyll content of the sediment of the whole lake.

Fig. 4 shows the depth variation of sedimentary cholorophyll (per gm. ignitable matter) for five of the lakes studied. The total depth of organic sediment in each lake was assigned a value of 100%. The depth of each level analyzed was expressed as a percentage of the total depth of organic sediment (surface = 0%). The lowermost, predominantly inorganic, sediments were purposely excluded because their organic content was low. The loss in ignition for inorganic sediments does not give a true measure of the organic content. Most of the ignition loss is due to loss of water from clays.

The important point to note in Fig. 4 is that sedimentary chlorophyll does not form a constant percentage of the sediment ignitable matter. The greatest concentration of sedimentary chlorophyll in the ignitable matter was found in the uppermost sediments of Redrock Lake, in the middle sediments of Muskee Lake and Lake Opinicon, and in the lowermost sediments of Bethany Bog. The meaning of the pronounced depth variations is not certain at the present time. Several different factors were probably operating to produce the observed variations. The productivity of the water body in question was doubtless one of these factors, but it is difficult to separate this influence from a number of others that may have come into play.

Discussion

The persistence of sedimentary chlorophyll in lake muds deserves comment. The instability of true chlorophylls (*a*, *b*, *c*, and others) under laboratory conditions is well known. It is therefore not too surprising to find the chlorophylls *a*, *b* and *c* have not been found in sediments, but it is perhaps surprising to find that the degradation products do persist and retain nearly the same absorption maximum as chlorophyll *a* (666 mμ for chlorophyll *a* in 90% acetone with our spectrophotometer). The primary reasons for the stability of the degradation products are probably three in number: subsurface

sediments are cold, devoid of light, and devoid of oxygen (1, 4). Heat, light, and oxygen all tend to destroy the chlorophylls. We have unpublished evidence to show that they are the primary factors in the destruction of sedimentary chlorophyll in solution, although not when in the mud (except for heat which does destroy sedimentary chlorophyll in its natural state). Surprisingly high amounts of free hexose and pentose sugars, as well as beta-carotene and myxoxanthin, have been recovered from lake sediments (18). As the study of the organic constituents of lake sediments continues it is likely that many more chemically and biologically "unstable" compounds will be found to be present. The cold reducing medium of a lake sediment offers ideal conditions for the preservation of organic compounds.

Our results do not indicate that no destruction of sedimentary chlorophyll has occurred or is occurring in the reduced sediments, but rather that the breakdown rate is slow. Synthesis of chlorophyll in subsurface sediments, if it occurs at all, must be quantitatively insignificant. Plants which synthesize chlorophyll in the dark (12) require a rich nutrient medium. From the chemical standpoint, sediments are rich in a variety of organic compounds, but many of these may be physiologically inert by virtue either of their structure or their location partly in the crystal lattices of clays.

Four separate occurrences of the destruction of organic compounds must be considered separately:

1. *In the water* where oxygen, light, temperature, and biological activity may bring about transformations of organic compounds both in living and dead cells.
2. *In the oxidized surface microzone* of the sediment where the above mentioned factors still operate.
3. *In reduced subaquatic mud* where destruction is probably slow (8).
4. *When subaquatic mud becomes transformed into a dry indurated rock.* Under condition of dryness, oxidation, and high temperatures, many compounds present in reduced subaquatic mud would be destroyed.

There are many difficulties in the interpretation of sedimentary chlorophyll data, apart from questions of destruction. Not all the sedimentary chlorophyll is derived from aquatic plants, for some plant parts are washed into the lake basin. A more serious difficulty is that the chlorophyll content of plant tissues varies with the tissue, the species, and the environment (6). When one adds to these difficulties the fact that the nature of sedimentary chlorophyll is almost entirely unknown and that three separate compounds are being measured as one, the situation appears hopeless. It must be realized however that many of these difficulties apply equally well to the interpretation of the results of plankton chlorophyll analyses, yet the plankton method has been widely used in both marine and inland waters with good success. As a quick measure of chlorophyll-bearing tissues in mud, the sedimentary chlorophyll method may find wide use. An investigation of the nature of sedimentary chlorophyll, together with extension of the work to other sediments, will provide a more exact basis for interpretation than is now possible.

Acknowledgments

The author is indebted to G. E. Hutchinson, Yale University, for criticism and guidance during the study. The Bethany Bog core was taken by D. A. Livingstone and the pollen analyses were done by H. Ignatius. The early work was supported by the National Science Foundation under a grant given to Professor Hutchinson. Later work was supported by the National Research Council of Canada.

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STUDIES ON RUBUS VIRUS DISEASES IN BRITISH COLUMBIA

II. BLACK RASPBERRY NECROSIS[1]

By R. Stace-Smith[2]

Abstract

Raspberry indexing in British Columbia demonstrated that several varieties are carrying a latent virus that induces mild symptons in other red raspberry varieties and severe symptoms on black raspberries (*Rubus occidentalis* L.). The name black raspberry necrosis is proposed for the disease and virus. The virus is transmitted by the aphid *Amphorophora rubi* Kalt. It may be acquired by the aphid after a half-hour feeding and transmitted with a two-minute transfer feeding. Most aphids lose their ability to transmit the virus within one and a half hours after leaving the source of inoculum.

Introduction

A program of indexing raspberry and blackberry plantings in British Columbia for the presence of latent viruses was commenced in 1950. At first root cuttings of healthy Washington red raspberry were used as indicator plants, and the varieties being tested were grafted to these plants. This grafting method proved slow and cumbersome. It was superseded by a method in which seedlings of North American black raspberry (*Rubus occidentalis* L.) were used as indicators and transmission was effected by means of the raspberry aphid, *Amphorophora rubi* Kalt.

This indexing program demonstrated that several commercial red raspberry varieties in British Columbia carry a latent virus that induces mild symptoms on other red raspberries and severe symptoms on black raspberries. The effects of that virus on tropical black raspberry (*Rubus albescens* Roxb.) and North American black raspberry (*Rubus occidentalis* L.) are reported here.

Literature

The first experimental interspecific transmission of a raspberry mosaic disease was reported by Wilcox and Smith (9) in 1924. They placed aphids (*A. rubi*) from red raspberries affected with mosaic on healthy black raspberries and found that the tips of the shoots of the inoculated plants wilted and died. At that time, only one raspberry mosaic was recognized and it was characterized on red raspberry by yellow distorted leaves with dark green elevations.

Two years later, in 1926, Dodge and Wilcox (7) noted that there appeared to be at least three mosaic diseases of black raspberry. One was characterized by a mild mottling of the foliage, another by a yellow-greening of the leaves

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and severe stunting of the affected plant; and a third, which they produced experimentally by inoculation from diseased red raspberry, caused necrosis of the petioles of the tip leaves and of the stem tip.

The observations of Dodge and Wilcox were verified the following year by Bennett (1), who applied the names mild mosaic, yellow mosaic, and red raspberry mosaic to similar types of mosaic found in black raspberry plantings in Michigan. He (2) later noted that, while yellow mosaic was distinct, there was no clear line of demarcation between the severe forms of mild mosaic and the milder forms of red raspberry mosaic. Consequently, in 1932, Bennett (3) classified the raspberry mosaics in Michigan into two general types, yellow mosaic and red raspberry mosaic. He noted that various isolates of red raspberry mosaic produced a graduated range of severity of symptoms when inoculated into black raspberry and he considered that strains of the virus accounted for the range. Bennett suspected that more than one virus was present in plants showing distinct mottling accompanied by green elevations and yellow depressions.

Working in New York, Rankin (8) also noted that red raspberry mosaic caused a wide range of symptoms in red and black raspberries. He considered the various symptoms explainable on varietal susceptibility and on environmental conditions rather than by variations of the virus as suggested by Bennett.

The latest work in America on the problem of the number of virus entities involved in red raspberry mosaic was reported in 1936 by Cooley (6). He transferred aphids from Columbian purple raspberry affected with a mild mosaic to healthy Cumberland and Plum Farmer black raspberries and found that the inoculated plants showed symptoms varying from mild mottling to severe mottling accompanied by necrosis. Cooley considered it improbable that more than one virus strain was concerned since the Columbian plants were uniformly affected with mild mottling, and he concluded that the varying reactions obtained were probably due to varietal differences and to the influence of environmental conditions.

The term "red raspberry mosaic" does not have a definite connotation in the American literature. Bennett (3) used the term to describe a general type of mosaic caused by more than one virus strain. His description of the disease on red raspberry does not include the striking mosaic consisting of yellow depressions and green elevations. Cooley (6) used the term for a disease of single virus origin and his description was broader in scope and included the striking mosaic symptoms.

The virus disease of *Rubus* here discussed appears to correspond with a portion of the red raspberry mosaic group described by Bennett (3) in Michigan. However, it appears unwise to retain the name "red raspberry mosaic" because of the indefinite connotation of this term. For this reason, the descriptive name black raspberry necrosis is being proposed for the disease and virus in British Columbia.

Methods and Materials

Methods were devised for indexing raspberry plants to determine whether or not they were carrying a latent virus. For indexing varieties that were naturally colonized by *Amphorophora rubi*, the following technique was employed. The lower surfaces of the young leaves near the tip of the current year canes were examined for aphid colonies and a leaf harboring a colony was detached and placed in a Petri dish containing a square of moistened blotting paper. At the laboratory, five aphids were transferred from the detached leaf to a healthy black raspberry seedling. After a transfer feeding of about one half hour on the seedling, the aphids were removed and destroyed.

For varieties on which aphid colonies were rare or absent in the field, the procedure was modified. A young leaf was detached from the plant being indexed and was placed in a moistened Petri dish. At the laboratory, aphids from a virus-free stock colony were given an overnight acquisition feeding on the detached leaf and then transferred to the indicator plant.

The use of aphids for indexing proved reliable for most American red raspberry varieties. However, the method was not dependable for indexing the European varieties Lloyd George and St. Walfried and these were indexed by grafting.

Black raspberry seedlings were also employed in trials to determine the vector relationships of black raspberry necrosis virus.

Symptoms

On Red Raspberry

Many varieties of red raspberries infected with black raspberry necrosis virus exhibit no visible symptoms and thus make the presence of the virus detectable only by indexing to a suitable indicator plant. The following varieties have been shown to carry the virus without showing symptoms: Newburgh, Latham, St. Regis, Malling Enterprise, Malling Promise, Seedling G, and Norfolk Giant.

Infected plants of the varieties Taylor and Washington exhibited a very mild mottling that appeared in the form of small chlorotic spots; such spots were usually more numerous along the secondary veins of the leaves (Fig. 1).

Red raspberry varieties infected with black raspberry necrosis virus that have been under observation for four years have shown no apparent decline in vigor.

On Himalaya Blackberry

The foliage of many Himalaya blackberry (*Rubus procerus* P. J. Muell.) plants showed a mild mottling similar to that observed on some red raspberry

FIGS. 1-4. Symptoms of black raspberry necrosis on:

FIG. 1. Leaf from infected Taylor red raspberry plant. FIG. 2. Leaf from infected tropical black raspberry (*Rubus albescens* Roxb.) plant. FIG. 3. Munger black raspberry (*Rubus occidentalis* L.) plant one week after inoculation: note downcurling of cane tip and wilting of tip leaves. FIG. 4. Leaf from tip of infected Munger black raspberry plant: note areas of necrotic tissue.

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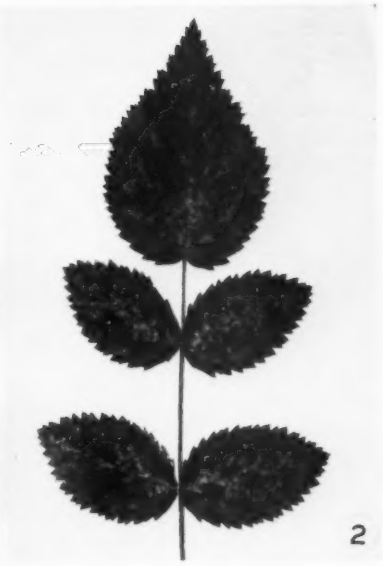
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varieties. The mottling appeared as small chlorotic spots on the leaves of both the primocanes and the fruiting canes. The symptoms, like those of red raspberry, were more pronounced on foliage produced during the cooler weather.

Graft transmissions from mottled Himalaya blackberry plants to Washington red raspberry, and aphid transmission to Munger black raspberry, demonstrated the presence of black raspberry necrosis virus in the Himalaya blackberry plants.

On Tropical Black Raspberry

Black raspberry necrosis virus was transmitted by aphids to tropical black raspberry (*Rubus albescens* Roxb.). Symptoms were first evident from six to eight days after inoculation, when small translucent light-green flecks appeared on the young expanding leaves at the tip of the inoculated plant. The older leaves at the base of the plant remained normal in appearance, but leaves produced at the tip and on axillary shoots of the inoculated cane were mottled to varying degrees (Fig. 2). The foliage of new suckers arising during the summer was also distinctly mottled. Canes were foreshortened and leaves were somewhat stunted.

In the year following inoculation, foliage of both the primocanes and fruiting canes was mottled and the plants were distinctly dwarfed.

On North American Black Raspberry

Black raspberry necrosis virus was transmitted to cuttings and open pollinated seedlings of North American black raspberry (*Rubus occidentalis* L.) var. Munger by the aphid *A. rubi*. The test plants were from four to six inches high and uniform in appearance when inoculated. Symptoms on the cuttings and the seedlings did not differ appreciably, except that the cuttings were uniform in symptom expression whereas the seedlings showed minor variations.

Symptoms were first evident from five to seven days after inoculation, when the tip of the inoculated plant appeared slightly bent. Within a day or two of the initial bending the tip was distinctly downcurled and brittle and the partially expanded leaves beneath the tip appeared wilted (Fig. 3). The wilting was generally unilateral, involving one of the basal leaflets and the lower part of the corresponding terminal leaflet. Then small flecks of necrotic tissue next appeared along the petiole, midrib, and secondary veins of the young unfolding leaves at the tip and on the wilted portion of the leaves beneath the tip. These flecks enlarged and coalesced resulting in diffuse areas of blackened necrotic tissue (Fig. 4). That portion of the partially mature leaves which did not become necrotic generally developed a yellow-green mottle or a distinct yellow cast.

Coincident with the development of necrosis of the leaves, necrotic flecks appeared along the leaf traces at the tip of the stem and gradually spread to the point where, about two weeks after inoculation, the cane tip was killed back two or three inches.

Wilting and necrosis were also evident in the axils of the basal leaves, where the expanding buds or short axillary shoots were completely killed. The immature buds in the axils of the leaves immediately beneath the necrotic cane tip appeared uninjured and, three to four weeks after inoculation, a shoot was produced from one of these axillary buds. Leaves on this shoot were stunted and some of them exhibited a light green mottle.

The severe necrosis of the stem tip and axillary buds could be considered a shock reaction since the young tissue formed after this initial reaction was not necrosed.

When seedlings less than three inches high were infected the necrosis extended to the hypocotyl and usually resulted in the death of the plants.

The symptom picture was very similar when the virus was transmitted by grafting instead of by aphids. The period between grafting and the appearance of initial symptoms, however, was from 16 to 20 days as compared with five to seven days for aphid inoculations.

Black Raspberry Necrosis Virus in Commercial Plantings of Red Raspberry in British Columbia

The principal center of raspberry production in British Columbia is the lower Fraser Valley region, where approximately 1400 acres are grown. Newburgh is the principal variety, accounting for approximately 1100 acres. Washington is next in importance with 150 acres, and the remaining acreage is made up of small plantings of such varieties as Cuthbert, Latham, Lloyd George, Willamette, and Taylor.

Commercial plantings of the various red raspberry varieties were surveyed to determine the incidence of black raspberry necrosis virus. Virus infection was readily determined by visual examination of field plantings of varieties that exhibited diagnostic foliar markings when infected with the virus. In those varieties that behaved as symptomless carriers, virus infection was determined by indexing to black raspberry seedlings. The status of the various symptomless and visibly infected varieties was as follows:

Newburgh.—All of 30 plants selected from seven commercial fields when indexed proved to be carrying black raspberry necrosis virus. This survey suggests that the original stock of this variety in British Columbia was infected and that subsequent commercial plantings are consequently totally infected.

Washington.—Several fields of this variety were surveyed and found to be virus free; however, one diseased plant was found in a mixed planting of Washington and Newburgh.

Other Varieties.—Small plantings of Cuthbert, Taylor, Latham, Lloyd George, Willamette, Preussen, Seedling G, Norfolk Giant, Malling Promise, Malling Enterprise, Malling Landmark, and St. Regis, were indexed. Willamette proved to be virus free; the other varieties were totally infected.

Vector Relationships

Acquisition Feeding Period

An experiment was conducted to determine the minimum time that an aphid must feed on the virus source before it acquires an infective charge of black raspberry necrosis virus. The source of inoculum was a Newburgh plant which had been indexed and had proved to be a carrier of the virus. Two groups of aphids were used, one of which was starved in a Petri dish for 16 hr. prior to feeding on the virus source; the other was not starved prior to the acquisition feeding.

Six aphids from each group were permitted to feed for 15 min. on the virus source before being transferred to black raspberry seedlings, one aphid per seedling, for a transfer feeding of 15 min. The aphids were then removed and destroyed. Other groups of starved and unstarved aphids were given acquisition feedings of 30 min., one hour, one and a half hours, two hours, two and a half hours, three hours, and six hours before being transferred to seedlings for a 15-min. transfer feeding. The results of these trials are given in Table I.

TABLE I

RESULTS* OF TRIALS TO DETERMINE THE LENGTH OF ACQUISITION FEEDING REQUIRED BY STARVED AND UNSTARVED APHIDS TO TRANSMIT BLACK RASPBERRY NECROSIS VIRUS TO *Rubus occidentalis*

	Duration of acquisition feeding							
Aphids	15 min.	30 min.	1 hr.	1½ hr.	2 hr.	2½ hr.	3 hr.	6 hr.
Starved (16 hr.)	0/6	1/6	2/6	3/6	3/6	4/6	4/6	4/6
Unstarved	0/6	1/6	2/6	3/6	2/6	5/6	3/6	4/6

* *Numerators indicate the number of transmissions. The denominators indicate the number of plants inoculated.*

The results indicate that the minimum acquisition feeding period for both starved and unstarved aphids is from 15 to 30 min., and that the ability of the aphids to transfer the virus increases if they remain for one hour to an hour and a half on the virus source. Longer acquisition periods do not noticeably increase efficiency of transmission.

Minimum Transfer Feeding

Experiments were conducted to determine the minimum time that viruliferous aphids must feed upon a healthy plant in order to transmit black raspberry necrosis virus. After a 24-hr. acquisition feeding, single aphids were transferred to black raspberry seedlings and permitted to feed for a given period. The transfer feeding times investigated were one, two, five, 10, 15, and 60 min.

The lapse of time between placement of aphids on the lower surface of the leaf of the test plant and the commencement of feeding (the prepenetration time) was taken into consideration. Aphids given short transfer feedings of one minute or two minutes were observed under a binocular microscope, and timing commenced when they began inserting their stylets into the leaf tissue. Aphids given a transfer feeding of five minutes or more were exposed to the indicator plant for an extra minute to compensate for the prepenetration time, since observations on many aphids indicated that they generally probe around for at least one minute before they insert their stylets.

The results, given in Table II, demonstrate that a two-minute transfer feeding enables aphids to transmit the virus to black raspberry seedlings. Also, the results indicate that a plant exposed to a viruliferous aphid for 15 min. is as likely to become infected as a plant exposed for a longer transfer feeding.

TABLE II

RESULTS* OF TRIALS TO DETERMINE THE LENGTH OF THE MINIMUM TRANSFER FEEDING REQUIRED BY APHIDS TO TRANSMIT BLACK RASPBERRY NECROSIS VIRUS TO *Rubus occidentalis*

	Duration of transfer feeding in minutes					
	1	2	5	10	15	60
Results	0/5	4/15	8/25	9/25	13/25	13/25

* *Numerators indicate the number of transmissions. The denominators indicate the number of plants inoculated.*

Persistence

The persistence of black raspberry necrosis virus in viruliferous aphids was investigated. Three different virus sources were used, namely: (1) Seedling G variety of red raspberry that was a symptomless carrier; (2) Taylor variety of red raspberry that showed mild mottling; and (3) Munger variety of black raspberry that was severely stunted and mottled. Aphids were given a 24-hr. acquisition feeding on these sources and were then transferred to black raspberry seedlings, one aphid being placed on each of five plants from each of the three virus sources. Aphids were allowed to feed for 15 min. on the first plant of the series, after which they were transferred individually to other black raspberry seedlings and permitted to feed for 30 min., one hour, three hours, and 24 hr. on other plants in the series. After the last feeding, the aphids were removed and destroyed. The results, summarized in Table III, indicate that the aphid loses its capacity to transmit the virus within two or three hours after leaving the source of inoculum.

In another experiment, more precise data were obtained on the persistence of black raspberry necrosis virus in the aphid vector. After an acquisition feeding of one day on an infected Washington red raspberry plant, 15 aphids

TABLE III

RESULTS* OF TRIALS TO DETERMINE THE ABILITY OF APHIDS TO RETAIN BLACK RASPBERRY NECROSIS VIRUS

Virus source	Duration of successive transfer feedings				
	¼ hr.	½ hr.	1 hr.	3 hr.	24 hr.
Seedling G	2/5	3/5	3/5	0/5	0/5
Taylor	1/5	2/5	2/5	0/5	0/5
Munger	1/5	4/5	2/5	2/5	0/5

* *Numerators indicate the number of transmissions. The denominators indicate the number of plants inoculated.*

were placed individually on black raspberry seedlings and permitted to feed for 30 min. Each aphid was then transferred to a second plant in the series and given a 30-min. transfer feeding. This procedure was repeated through a series of eight plants, after which the aphids were destroyed. The performance of each aphid is given in Table IV.

TABLE IV

RESULTS OF TRIALS TO DETERMINE THE ABILITY OF INDIVIDUAL APHIDS TO RETAIN BLACK RASPBERRY NECROSIS VIRUS

Test aphid number	Duration of successive transfer feedings (hr.)								Total
	½	½	½	½	½	½	½	½	
1	+	+	−	−	−	−	−	−	2
2	−	+	+	−	−	−	−	−	2
3	+	−	−	−	−	−	−	−	1
4	+	+	+	−	−	−	−	−	3
5	−	−	−	−	−	−	−	−	0
6	−	+	−	−	−	−	−	−	1
7	+	+	−	−	−	−	−	−	2
8	−	+	−	−	−	−	−	−	1
9	+	+	+	+	+	+	+	−	7
10	+	+	+	−	−	−	−	−	3
11	+	+	+	−	−	−	−	−	3
12	−	−	−	−	−	−	−	−	0
13	+	+	−	−	−	−	−	−	2
14	+	−	−	+	+	−	−	−	3
15	+	−	−	−	−	−	−	−	1
Total	10	10	5	2	2	1	1	0	31

Black raspberry necrosis virus was transmitted to at least one plant by 13 of the 15 aphids. Ten aphids transmitted the virus to the first series of plants, and seven of these 10 aphids transmitted the virus to the second plant in the series. A sharp decrease in transmission to the third and fourth series of plants indicated that most aphids lose their capacity to transmit the virus within one and a half hours after leaving the source of inoculum. One of the aphids, however, retained an infective supply of the virus longer than three hours but less than three and a half hours.

Discussion

The symptoms produced in black raspberry seedlings by the various isolates of viruses which were latent or mild in red raspberries and Himalaya blackberries were quite uniform, and it seems fair to consider that a single virus entity was involved. This situation is in contrast to the findings of Bennett (3) who described a wide range of symptoms in black raspberry from different isolates.

The disease corresponds to Bennett's description of medium severe red raspberry mosaic. The term "red raspberry mosaic", however, does not have a definite connotation in the American literature, and it appears unwise to apply this name to the British Columbia disease. For this reason, the descriptive term "black raspberry necrosis" is proposed.

Black raspberry necrosis appears to have a number of features in common with raspberry leaf mottle described in Scotland by Cadman (4, 5). However, Cadman considered leaf mottle to be etiologically distinct from other previously described virus diseases and the British Columbia disease appears to correspond with a portion of the red raspberry mosaic group described in 1932 by Bennett (3).

Acknowledgments

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THE SEASONAL VARIATION IN CHEMICAL COMPOSITION OF *MACROCYSTIS INTEGRIFOLIA* AND *NEREOCYSTIS LUETKEANA* IN BRITISH COLUMBIA COASTAL WATERS[1]

By D. J. Wort

Abstract

Macrocystis integrifolia and *Nereocystis luetkeana* were collected at Port Hardy and Vancouver, B.C., respectively, at monthly intervals October 1949 through September 1951. Analyses of fronds and of stipes were made for dry weight, ash, total nitrogen, total phosphorus, sulphur, copper, zinc, iron, ether-solubles, algin, mannitol, fucoidin, and laminarin. Generally speaking the fronds had a higher content of all substances measured than did the stipes, and the content of the two species of algae showed parallel variation but differed in values. Dry weight and ash were at maxima in late fall and winter and at minima in April and May. During March total nitrogen, ether-solubles, mannitol, fucoidin, and laminarin were at their highest concentration. The plants in July contained the minima of total nitrogen, phosphorus, and ether-solubles. The maximum for phosphorus occurred in December and during this same month fucoidin was at minimum. The variation of sulphur, copper, zinc, iron, and algin was such that well marked minima and maxima were not evident.

The steadily rising interest in seaweeds as sources of algin, mannitol, fucoidin, and laminarin and the use of algae as human, stock, and poultry food has once again underlined the necessity of additional data on the chemical composition of marine algae and the changes in this composition attributable to season, habitat, depth of growth, and other environmental factors.

The first systematic investigation into seasonal variation in the chemical composition of seaweed was the work of Lapicque in 1919 (15). A number of workers in various countries conducted analyses of marine algae collected at intervals during the year—Colin and Ricard in France (8, 9), Lunde in Norway (17), Atsuki and Tomoda in Japan (1), Kizevetter (14) and Vedrinskii (24) in Russia, and Dillon in Eire (10). These investigators included dry weight, ash, algin, mannitol, laminarin, fucoidin, cellulose, and nitrogen in their studies. Black and his associates (2, 3), beginning in 1948, systematically studied seasonal variation in the chemical composition of a considerable number of marine algae of the British Isles.

Investigations of the chemical composition and its variation in Canadian algal material have been confined to the eastern coast species primarily. Butler (6) and Macpherson and Young (18) have studied the variation in composition of *Chondrus crispus*, *Fucus vesiculosus*, *F. evanescens*, and *Ascophyllum nodosum* collected at various points on the Atlantic coast. The data on chemical composition of algae on the Canadian Pacific Coast are few and none were obtained for collections made over a period of time greater than two months.

Macrocystis and *Nereocystis* are present in very large quantities on the western coast and are of real, though potential, economic importance. Rose (21) evaluated various brown seaweeds, occurring on Canadian coasts, as a

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source of alginate. The following ranking is in decreasing suitability as sources: *Laminaria digitata*, *Nereocystis luetkeana*, *Macrocystits integrifolia*, *Ascophyllum nodosum*, *Fucus vesiculosus*. The first three species are equal to seaweeds used commercially elsewhere.

The algal investigations of the British Columbia Research Council (4, 5) have emphasized the discovery and appraisal of commercially useful beds of marine plants and to a limited extent the chemical evaluation of representative samples collected during the field surveys. Their main survey was carried out in the late summer of 1946 and covered the northern coast from Wales Island, north of Prince Rupert, down to Seymour Narrows and Yuculta Rapids at the northern end of the Strait of Georgia. The chemical analyses published by the Council concern plants collected in August 1946 (4) and in the period July–August 1947 (5).

Macrocystis occurs on the west coast of both Americas, on the lower eastern coast of South America and encircles the Antarctic. It is found in the southern areas of Africa, Australia, and New Zealand. The species growing in British Columbia waters has been identified as *M. integrifolia* Bory, on the basis of holdfast characteristics. These plants differ ecologically as well as morphologically from *M. pyrifera* of California. *M. integrifolia* reaches a larger size and grows in shallower water than does *M. pyrifera*.

In general, *Macrocystis integrifolia* occurs along the outer sections of the coast in fringes of varying width, but is not found in the Strait of Georgia or in the inner passages. The plants are attached to large rocks by holdfasts, composed of numerous haptera, with such firmness that the plants remain attached except where there are extremely rapid currents and strong wave action. The beds are usually protected from strong wave action by reefs or by wide fringes of *Nereocystis*. They are found at depths varying between 2.5 ft. above and 20 ft. below zero tide level, with the greatest density at 15 ft. or less. Regions with a density of about 1.022 (at 15° C.) are preferred. The ordinary summer temperatures of the water in which the plants grow vary between 10° and 12° C. (12).

The life history involves an alternation of a large sporophytic generation with microscopic gametophytes. The sporophyte grows to a length of 60 ft. or more and has a much branched holdfast from which arise several stipes bearing numerous fronds with or without pneumatocysts. The free swimming spores, produced by the sporophyte, germinate and develop into microscopic thread-like male and female gametophytes. Following fertilization the egg develops into the large sporophyte. Whereas the greater part of the sporophyte may not survive the winter storms, the basal portion persists and may initiate new branches for several years. It is thus to a degree perennial.

The sporophyte must be about 10 months old to have harvestable stipes. Four to six feet below the surface appears to be the most desirable depth at which to harvest. The harvestable weight of individual plants varies between 12 and 100 lb., but when the ratio of size to abundance is taken into account, the density per cubic unit remains fairly constant (4).

Nereocystis luetkeana (Mert.) Post. and Rupr. is fairly widely distributed along the whole coast of British Columbia. The type of ocean bottom on which it grows is the same as for *Macrocystits*—large stones and rough-surfaced rocks to which the branched haptera anchor the plant. *Nereocystis* is commonly found in passages which have a strong tidal current and it can also withstand the extreme wave action of the exposed coast. Plants in the northern regions were found ranging from low tide level to 40 ft. of water and varying in harvestable weight from five to 22 lb. On the average there is about 3.5 times more weight in the fonds than in the upper six feet of stipe. Encrustation with the bryozoan *Membranipora* is common and various filamentous algae, including *Polysiphonia*, may grow plentifully on the large pneumatocyst and the stipe (4, 22).

Nereocystis is usually harvested from a barge equipped with a mower for cutting the plants at a depth of six to eight feet. The harvesting period would probably be between late July when the spores have matured and late September when the plants begin to break up (4).

Nereocystis luetkeana passes through a life cycle comparable to that of *Macrocystis*. The sporophytic generation, represented by the large kelp plant, produces patches of sporangia on its fronds. When mature, these patches loosen and drop out. As many as 50 fronds may be produced and may reach a length of over 26 ft. *Nereocystis* is in the main annual, and though some plants persist until the next spring and early summer these are in the minority.

Experimental

Collection of Material

Macrocystis

An area on the northern coast of Vancouver Island just east of Thomas Point near Port Hardy (lat. 50° 45′ N., long. 127° W.) was selected for collection since *Macrocystis* was plentiful, several studies had been carried on there (4, 22), and facilities were available for collection and surface drying prior to shipment to the laboratory. A boat was used to harvest attached plants at low tide on or as near as possible to the 24th of each month from October 1949 through September 1951. The portion of the plant taken included the major portion of the stipe with its fronds. During the winter months (beginning in October) the fonds were somewhat broken and scarce but by March and April they had grown to a length of 18 in.

The material, protected against the leaching action of rain, was taken to a shed and spread on nets to surface dry. Fifty pounds of the plants were packed in sacks and shipped by boat to Vancouver for final drying and analysis. The usual time lapse from collection to arrival at the laboratory at the University of British Columbia was three days. The seaweed appeared in excellent condition when received and although the composition may have been influenced by respiration in the time between collection and final drying this time lag could not be avoided. Obviously moisture percentage could not be accurately determined with this material.

Nereocystis

Plants of *Nereocystis* were collected on the 17th of each month, or as near as possible to that date, from October 1949 through September 1951 at Brockton Point, Vancouver, B.C. (lat. 49° N., long. 123° W.). The upper six feet of stipe, together with its fronds, was harvested by boat. Only attached plants were collected. The material was protected against rain and taken immediately to the laboratory for drying. The fronds of the mature plants were reduced to ribbons by January and the algae were very scarce during March and April. By May the new plants had produced fronds 1½ to 4 ft. in length.

Washing, Drying, Grinding

In the laboratory the *Macrocystis* and the *Nereocystis* plants were washed in running tap water for a standard period of five minutes to remove extraneous matter and most of the surface salt. Advantages gained by the washing were thought to outweigh the possible leaching effects. The fronds, separated from the pneumatocysts, were spread on string nets and allowed to dry at room temperature for 48 hr. They were then crushed, thoroughly mixed, and stored in sacks. The stipes and pneumatocysts were chopped into small pieces and spread out in trays to dry for 48 hr. The dried pieces were then thoroughly mixed and stored in stoppered bottles at room temperature.

One-hundred-gram lots were withdrawn from the air dried fronds or stipes, oven dried at 95° C., ground in a hammer mill to pass through a 100-mesh sieve, and stored in bottles until needed. The fine powder was again dried in the oven and allowed to cool over calcium chloride in desiccators before aliquots were taken for the various analyses. Every effort was made to ensure representative sampling.

Methods of Analysis

Dry weight and moisture determinations were done on three separate 75-gm. lots of fresh *Nereocystis* fronds and stipes by drying to constant weight at 95° C. As mentioned above, moisture determinations of *Macrocystis* were not attempted.

The ash content of fronds and of stipes was found by igniting three 5-gm. aliquots of oven-dry powder in an electric muffle furnace at approximately 600° C.

Total nitrogen was determined by the standard Kjeldahl method modified to include nitrates (16).

Total phosphorus was ascertained by wet ashing duplicate 0.1 gm. aliquots with concentrated sulphuric acid and hydrogen peroxide and measuring phosphorus colorimetrically by a modification of the method of Sumner (23).

Sulphur determinations were made by the method given in Loomis and Shull (16).

Copper, zinc, and iron measurements were kindly made by Warren and Delavault using methods employed by them in their biogeochemical studies (25, 26).

Ether-soluble substances were determined by extracting duplicate 5-gm. aliquots of oven-dry powder with ether in a Soxhlet apparatus for 48 hr.

Algin, expressed as sodium alginate, was measured by the method of Percival and Ross (19).

Mannitol, fucoidin, and laminarin were ascertained by methods given by Cameron *et al.* (7).

Results

All percentages, with the exception of dry weight, are based on oven-dry weight.

Dry Matter

The dry matter content of fronds and stipes of *Nereocystis* was relatively constant throughout the two year period. The two year average for fronds was 6.40% (Table I), with a range from 4.71 to 9.63%, and for stipes 6.88% with a range from 4.37 to 8.93%. The values were at maximum during the early months of the year when the plants were mature and were gradually breaking up. The new plants of April–May showed a definite drop in the percentage of dry matter. The periods of maximum and minimum coincide with those reported by Black (2) for British Laminariae and by Macpherson and Young (18) for Canadian east coast Fucaceae. The dry matter content of the entire plant of *N. luetkeana* collected at Pacific Grove was given as 8.3% by Hoagland (11). This percentage falls within the ranges of values recorded above.

TABLE I

DRY WEIGHT OF *N. luetkeana* EXPRESSED AS PER CENT OF FRESH WEIGHT

Year	Month of collection	Dry weight, %		Year	Month of collection	Dry weight, %	
		Fronds	Stipes			Fronds	Stipes
1949	Oct.	6.26	8.93	1950	Oct.	5.50	4.64
	Nov.	6.44	8.74		Nov.	6.62	7.30
	Dec.	6.74	7.66		Dec.	5.78	6.15
1950	Jan.	7.17	7.97	1951	Jan.	5.58	7.60
	Feb.	8.39	7.99		Feb.	6.66	6.18
	Mar.	9.63	7.72		Mar.	7.83	7.31
	Apr.	7.18	7.79		Apr.	6.45	5.22
	May	6.05	5.90		May	5.29	4.37
	June	6.68	8.19		June	5.48	7.86
	July	6.60	7.31		July	5.84	5.75
	Aug.	6.58	7.71		Aug.	4.71	4.58
	Sept.	4.86	5.74		Sept.	5.11	6.12
Year average		6.90	7.64	Year average		5.90	6.12
Two year average		6.40	6.88				

Ash Content

In Fig. 1 the ash contents of the algae are given graphically. While the percentage of ash is higher in *Nereocystis* than in *Macrocystis* the values have features in common. The stipes contain a higher percentage of ash than do the fronds and maxima in late fall and in winter are evident in both fronds and stipes of *Macrocystis* and in the fronds of *Nereocystis*. The range in va lues over the two year period for *Nereocystis* fronds was 32.0 to 47.5% and for stipes 45.5 to 63%. These values exclude one very low point found in the frond and in the stipe data. Black's (2) values for the holdfast (39.0%) and stipe (40.8%) of *N. luetkeana* from San Juan, Wash., were below the range of values reported here for the upper six feet of the stipe of this species.

The range for *Macrocystis* fronds was 19.0 and 40.0% and for stipes 32.5 to 52.0%. With the appearance of new growth in the early spring the ash content declined. The periods of maximum ash content and maximum dry weight coincide with those of greatest rain water drainage from the coastal slopes.

These results are similar to those of Macpherson and Young (18) for *Fucaceae* collected at Halifax in 1951, but are unlike those of Black (2) for *Laminariae*. Maxima occurred in the spring and minima in the autumn in this latter case. Butler (6) found that the ash content of *Chondrus crispus* of the east coast of Canada rose in spring and summer.

Total Nitrogen

Fronds of both *Nereocystis* and *Macrocystis* contained a higher percentage total nitrogen than did their stipes. Although the variation of values was greater in *Macrocystis* than in *Nereocystis* the abundance of nitrogen changed but little from month to month. The values over the two year period varied from 1.47 to 3.7% in the fronds and from 0.6 to 1.7% in the stipes of *M. integrifolia*. The ranges of values in *Nereocystis* fronds and stipes were 2.5 to 3.6% and 1.25 to 2.1%, respectively. The nitrogen percentages are shown in Fig. 2. Maximum values were measured in the *Macrocystis* collected during March to May, while minimum values were obtained in the July material. There were indications of a secondary minimum in December for both species.

The rise and fall of total nitrogen and its relatively small fluctuations are in agreement with those of Black (2) and Macpherson and Young (18). Nitrogen values reported by Hoagland (11) for *M. pyrifera* collected at Pacific Grove show fronds and stipes with average contents of nitrogen of 2.65 and 1.14%, respectively. *Nereocystis* frond and stipe values were 2.00% and 1.20%. Black measured 1.22% total nitrogen in the stipe of *N. luetkeana* from Pacific Grove. These are comparable to the values reported here.

Total Phosphorus

The total phosphorus values, shown in Fig. 3, were quite constant during the two year period of collection. The range of values for both algae was from 0.3 and 1.3%, with the large majority of values falling between 0.4

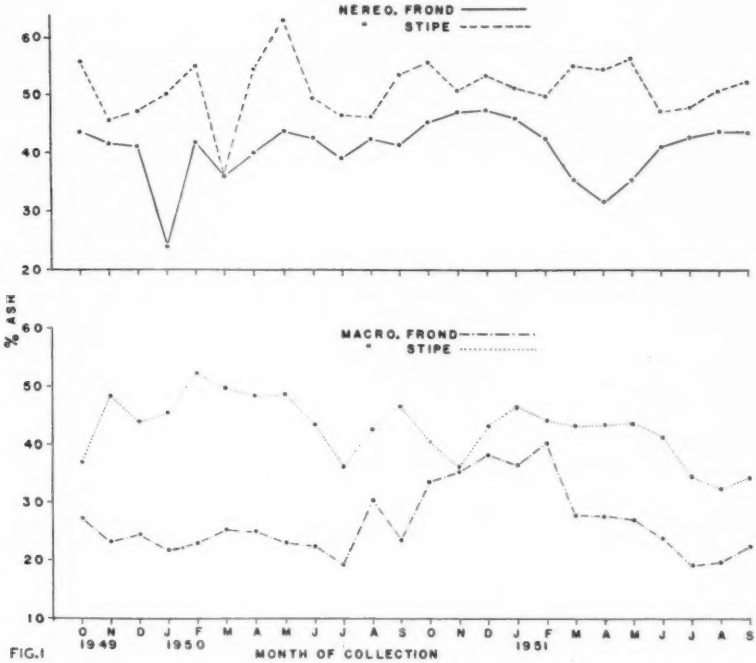

FIG. 1

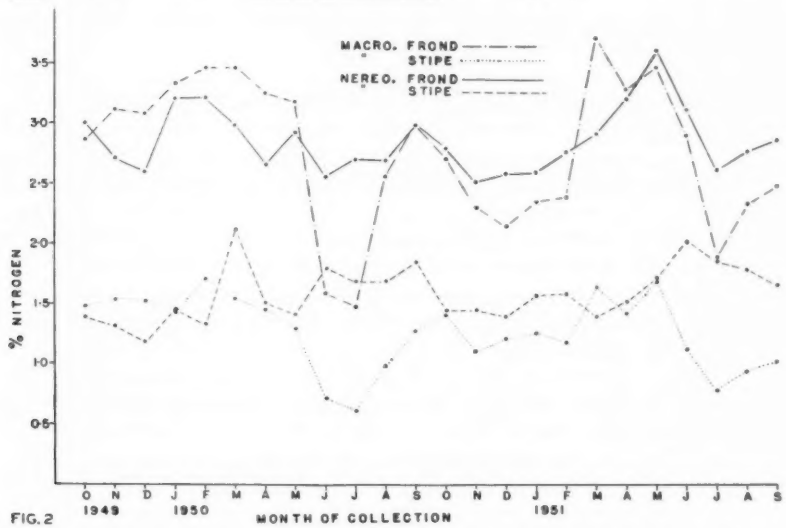

FIG. 2

FIG. 1. Seasonal variation in ash content of fronds and stipes of *M. integrifolia* and *N. luetkeana*. FIG. 2. Seasonal variation in total nitrogen content of fronds and stipes of *M. integrifolia* and *N. luetkeana*.

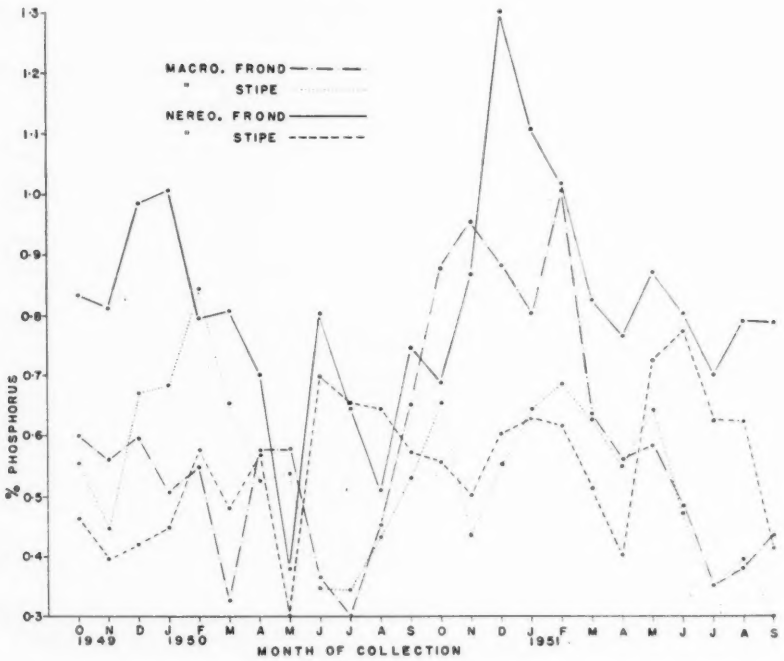

FIG. 3. Seasonal variation in total phosphorus content of fronds and stipes of *M. integrifolia* and *N. luetkeana*.

and 0.8%. The maximum phosphorus content of *Nereocystis* fronds was measured in December–January, while the minimum for *Macrocystis* occurred in July.

Productivity of the sea depends primarily on the availability of nitrate and phosphate. The content of these ions in the water of San Juan Channel, Wash., where *N. luetkeana* predominates, is not subject to the marked seasonal variation which is characteristic of the sea water of the British Isles. The phosphate and nitrate figures are about four times higher in this Channel than in British waters, and the concentrations are always sufficient for plant growth. The maxima occur in December–January and the minima in August (20). Phosphate fluctuation in the Strait of Georgia, measured by Hutchinson *et al.* (13) follows a similar pattern. These periods are reflected in the phosphorus content of the *Nereocystis* collected at Vancouver but the nitrogen content of these plants showed little variation with season.

Sulphur

The sulphur percentages are not listed since they varied between such narrow limits and maxima and minima were not apparent. The average

sulphur content of *Macrocystis* fronds was 1.52% with 0.76 and 2.35% being the limits of variation. The average for the stipes of this plant was 1.11%, with limits of variation being 0.68 and 2.08%. *Nereocystis* fronds contained an average of 1.23%, ranging from 0.89 to 2.25%, and the stipes contained an average of 1.01% with the limits being 0.53 and 1.69%. These data are very similar to those published by Hoagland (11) for *M. pyrifera* and *N. luetkeana*.

Copper, Zinc, Iron

The content of copper, zinc, and iron is given in Table II. The variation is such that selection of periods of maximum values that would hold for the two years of collection is very difficult. The two year averages, expressed as p.p.m., followed in brackets by the limits of values, were as follows: *Macrocystis:* copper—fronds 59 (14–243), stipes 22 (4–87); zinc—fronds 97 (14–335), stipes 21 (10–34); iron—fronds 562 (100–3250), stipes 125 (30–400). *Nereocystis:* copper—fronds 46 (3–140), stipes 29 (7–70); zinc—fronds 424 (90–2800), stipes 74 (7–310); iron—fronds 153 (4–420), stipes 124 (30–360). The copper and zinc maxima are approximately twenty times, and the iron maximum in *Macrocystis* fronds ten times the concentration found in the leaves and stems of gymnosperm and angiosperm trees and shrubs growing on the Pacific coast (25, 26).

Ether-solubles

In both species the percentage ether-solubles was higher in the fronds than in the stipes. In the latter there was little variation from month to month. The range of values, given in Fig. 4, for *Macrocystis* fronds was 0.6 to 1.95%, and for its stipes 0.4 to 1.25%. *Nereocystis* frond values ranged from 1.4 to 4.4% while those of the stipes varied between 0.3 and 1.2%. The highest values in the fronds of *Nereocystis* were obtained in March of both years and while the peak is less pronounced in *Macrocystis* it would appear that there was a tendency toward a March maximum also. A low in *Nereocystis* was apparent in the June–July collections. The percentage of ether-solubles in *M. pyrifera* collected at San Diego and at Pacific Grove was 0.34% and 0.40%, respectively, and in Pacific Grove *Nereocystis* 1.06% (11).

Algin

The algin content of *Macrocystis* and *Nereocystis*, expressed as sodium alginate, is given in Fig. 5. Over the two year period the algin content of the fronds was slightly greater than that of the stipes but little variation from month to month appeared. The percentage of algin in *Macrocystis* fronds ranged from 14.5 to 36%, and in the stipes from 15.5 to 30%. The fronds of *Nereocystis* contained from 16 to 32.5%, and the stipes 13 to 39.7%. The algin content of these two species is higher than that reported by Hoagland (11) for *M. pyrifera* (13.6 to 24.5%) and for *Nereocystis* (13.6 to 14.4%) growing in California waters. The well marked maxima and minima of algin

TABLE II

COPPER, ZINC, AND IRON CONTENT OF *M. integrifolia* AND *N. leutkeana* EXPRESSED AS PARTS PER MILLION OF OVEN-DRY WEIGHT

Year	Month of collection	Copper		Zinc		Iron	
		Frond	Stipe	Frond	Stipe	Frond	Stipe
M. integrifolia							
1949	Oct.	25	31	139	21	400	60
	Nov.	69	12	85	18	450	80
	Dec.	16	9	31	13	260	60
1950	Jan.	27	17	335	25	230	80
	Feb.	15	7	320	16	350	90
	Mar.	14	4	233	15	270	60
	Apr.	26	11	116	8	1250	130
	May	243	42	226	10	270	140
	June	34	58	64	15	120	160
	July	135	87	202	18	110	120
	Aug.	87	15	30	13	160	250
	Sept.	32	8	20	10	170	60
Year average		60	25	151	15	337	108
Two year average		59	22	97	21	562	125

Year	Month of collection	Copper		Zinc		Iron	
		Frond	Stipe	Frond	Stipe	Frond	Stipe
1950	Oct.	39	8	20	14	1100	120
	Nov.	36	4	14	11	1500	170
	Dec.	35	8	19	12	1750	400
1951	Jan.	57	7	22	13	3250	280
	Feb.	45	17	42	23	300	100
	Mar.	120	20	54	29	200	200
	Apr.	82	28	31	19	150	80
	May	75	17	22	49	100	80
	June	60	30	104	39	300	40
	July	41	34	75	47	150	30
	Aug.	37	26	80	39	400	50
	Sept.	75	26	34	21	250	150
Year average		57	19	43	26	788	142

TABLE II—*Concluded*

COPPER, ZINC, AND IRON CONTENT OF *M. integrifolia* AND *N. luetkeana* EXPRESSED AS PARTS PER MILLION OF OVEN-DRY WEIGHT—*Concluded*

Year	Month of collection	Copper		Zinc		Iron	
		Frond	Stipe	Frond	Stipe	Frond	Stipe
N. luetkeana							
1949	Oct.	36	15	2800	97	90	300
	Nov.	41	17	1250	62	100	30
	Dec.	24	24	150	57	60	40
1950	Jan.	41	22	440	50	240	60
	Feb.	35	29	580	67	60	80
	Mar.	20	34	440	310	100	100
	Apr.	16	12	260	70	320	80
	May	28	32	370	37	60	50
	June	32	44	560	7	4	100
	July	65	47	300	53	90	360
	Aug.	140	70	840	62	420	280
	Sept.	25	7	90	170	80	60
Year average		42	29	673	87	135	128
Two year average		16	29	424	74	153	124
1950	Oct.	3	29	120	87	220	100
	Nov.	29	18	110	42	100	80
	Dec.	53	13	130	48	140	100
1951	Jan.	36	14	160	45	200	140
	Feb.	56	39	135	39	200	250
	Mar.	52	34	170	44	200	100
	Apr.	73	34	143	44	250	100
	May	76	28	420	117	150	80
	June	59	37	184	63	150	70
	July	49	32	154	73	100	120
	Aug.	52	30	143	81	120	150
	Sept.	56	41	234	44	200	150
Year average		50	29	175	61	169	120

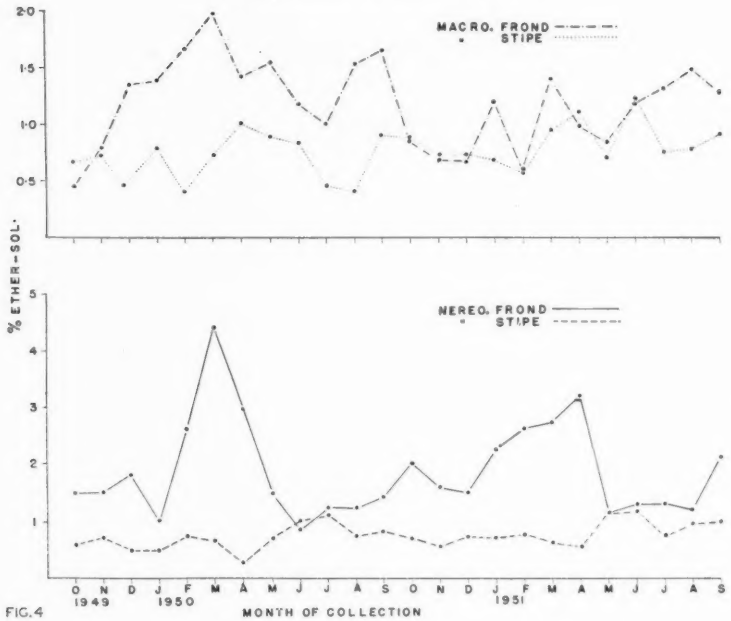

FIG. 4

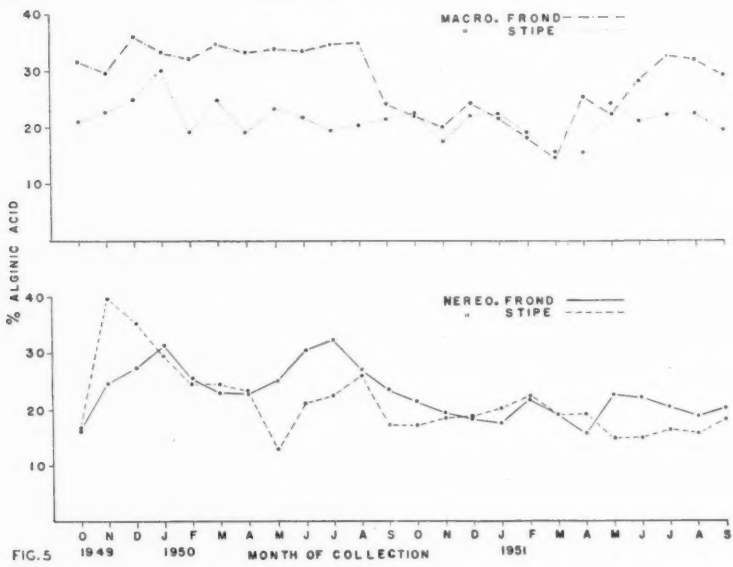

FIG. 5

FIG. 4. Seasonal variation in ether-solubles content of fronds and stipes of *M. integrifolia* and *N. luetkeana*. FIG. 5. Seasonal variation in algin content of fronds and stipes of *M. integrifolia* and *N. luetkeana*.

content of British Laminariae (2) were not obtained in the two British Columbia kelps and in this respect the results were more similar to those reported by Macpherson and Young (18) for Halifax Fucaceae.

M. integrifolia was collected at Deer Island, Hardy Bay, Hope Island, Aristazabal Island, Banks Island, and Melville Island in August 1946 by the British Columbia Research Council and analyzed for algin. The average algin content of the fronds was 21.75% (the values ranged from 14.1 to 25.1%) and of the stipes 26.14% (with 19.3 and 27.0% as the limits). Values for *N. luetkeana* were 18.8% and 24.4% for fronds and stipes, respectively. The Council also examined samples of *M. integrifolia* and *N. luetkeana* collected at Deer Island near Port Hardy, B.C., at intervals from July 1 to the end of August 1947. All plant parts were mixed for each analysis, hence values for fronds and for stipes were not obtained. The percentages of algin, expressed as sodium alginate, on an oven dry basis (as are the values reported in this paper) were: *M. integrifolia:* July 1, 20.9%; July 16, 20.1%; July 29, 26.7%; Aug. 11, 24.7%; Aug. 25, 24.4%. *N. luetkeana:* July 17, 20.3%; July 29, 19.5%; Aug. 11, 21.0%. It was reported that the quality of the algin improved somewhat as the season advanced.

Mannitol

The data indicate that the mannitol distribution in *Nereocystis* and *Macrocystis* is quite different (Fig. 6). In the former there is a higher percentage and a greater variation in the frond, while in the latter the higher content and greater variation are found in the stipes. The mannitol content of *Nereocystis* fronds remained between 3 and 6% for 19 of the 24 months and rose to between 9 and 12% during February–April 1950 and March–April 1951. Stipe mannitol remained relatively constant (1 to 3%) for 23 of the 24 months, and rose to 9.4% in March 1950 only. The range of mannitol content of *Macrocystis* fronds, 1 to 10%, had high points in June and July 1950 but these did not recur in 1951. The range in the stipes, 1.5 to 26%, showed four peaks, primary ones in June–August, and secondary ones in October–November. A variation showing maxima in spring and summer with a secondary maximum about November is similar to that reported by Macpherson and Young (18) but unlike the autumn maximum and spring minimum found by Black (2).

Fucoidin

Fig. 7 shows the fucoidin values expressed as percentage of fucose. The fronds of both algae have a higher fucoidin content than do the stipes but the amounts vary little with time. The limits of variation in *Macrocystis* fronds are 4.1 and 6.25%, and in the stipes 3.1 and 5.5%. In *Nereocystis* the values of fucoidin in the fronds vary between 3.1 and 7.0% with the maximum measured in March of both years and the minimum occurring in December. The stipe values, 2.3 to 3.8%, exhibit no well marked peaks.

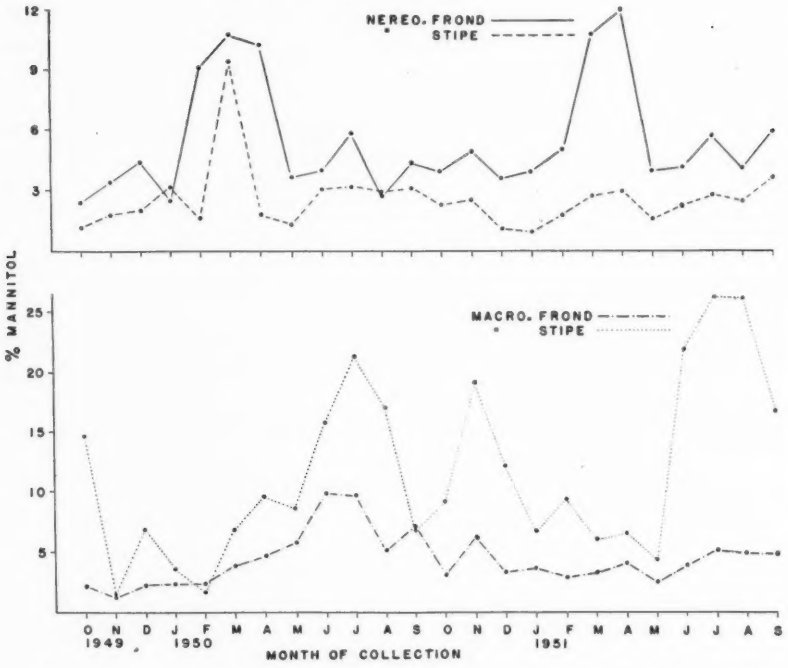

FIG. 6. Seasonal variation in mannitol content of fronds and stipes of *M. integrifolia* and *N. luetkeana*.

Laminarin

Laminarin percentages, shown in Fig. 8, fall within a narrow range of values. A maximum is suggested for early spring, followed by lower values for the rest of the year. The laminarin contents are: *Macrocystis* fronds 0.3 to 1.95%, stipes 0.4 to 1.5%; *Nereocystis* fronds 1.05 to 3.1%, and stipes 1.25 to 3.1%. These values are similar in magnitude and distribution to those obtained for Fucaceae on the east coast (18) but are unlike those measured by Black (2) in Laminariae.

Discussion

In the search for the reasons for fluctuations in composition of algae it must be borne in mind that these plants depend upon light as a source of energy for the photosynthetic process and upon the water for dissolved carbon sources and nutrients generally. Changes in light intensity and duration due to season or depth of immersion; in nutrient supply caused by thermocline, currents, proximity of river mouths, and depletion by other organisms; in temperature; in oxygen; in salinity; in pH; and so on make their impression on the metabolism and growth of the algae. Black and Deward (3) have

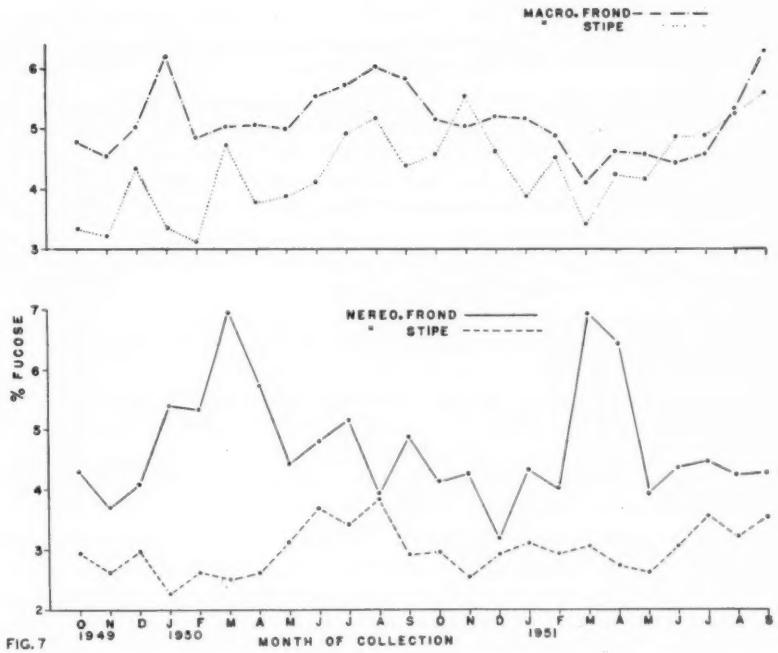

FIG. 7

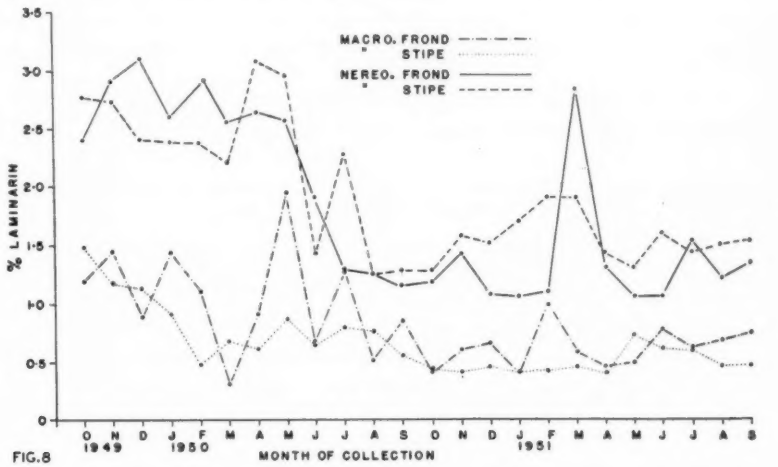

FIG. 8

FIG. 7. Seasonal variation in fucoidin content of fronds and stipes of *M. integrifolia* and *N. luetkeana*. FIG. 8. Seasonal variation in laminarin content of fronds and stipes of *M. integrifolia* and *N. luetkeana*.

related environmental factors with the composition and metabolic activities of various British Laminariae, and Macpherson and Young (18) have attempted to correlate their findings with environmental factors on the eastern coast of Canada.

Photosynthesis by brown algae is much less understood than that of the green algae and higher plants but the essentials of the process may well be the same. It is believed that phosphorylated mannose compounds are involved in the sequence and that the hexahydric alcohol, mannitol, is a primary product of photosynthesis. Alginic acid, the cell wall constituent, occurs as various metal salts and consists of *d*-mannuronic acid residues in 1–4 linkages. These residues suggest its genesis from or through mannitol. Algin exhibits the geometric pattern of cellulose rather than of starch, is analogous to pectin, and exhibits strong hydrophilic properties. The fronds of *M. integrifolia* and *N. luetkeana*, which are the chief centers of photosynthesis of these plants, have their highest mannitol content during the spring and early summer. It is at these times that photosynthesis is most active. Similar results were obtained by Macpherson and Young (18) but Black (2) reported that mannitol is at minimum in the fronds of Laminariae in the spring. The alginic acid content of the western coast algae, while lacking marked maxima, resembles that of the British Laminariae in that there is a tendency for a rise in content in early summer. Maximum values between November and March were recorded by Macpherson and Young (18).

Laminarin, which is the water soluble reserve carbohydrate of kelps, is analogous in function to starch, but differs from the latter in that its glucopyranoside residues have a 1–3 instead of the 1–4 linkage present in starch. It has been suggested that laminarin is derived from mannitol. A reciprocal relationship of these two compounds is not evident in the analyses of *Macrocystis* and *Nereocystis* and it is not apparent in Fucaceae (18) or in Laminariae (2). Contrawise a parallel change in concentration occurs.

Fucoidin is a water soluble sulphuric acid ester of a polysaccharide which on hydrolysis gives pentoses and methyl pentoses, particularly fucose. Its, function is not known but it has been suggested that its base-binding capacity, along with that of algin, is of importance in determining the concentration of total ash. In *Macrocystis* and *Nereocystis*, however, total ash concentration and algin and fucoidin content show no parallel variation. Ash and fucoidin are present in an inverse relationship and the levels of algin also suggest an inverse relationship to ash.

Nitrate and phosphate are two of the most imporant factors in the growth of seaweeds. Upon reduction the nitrate is involved in the amination of organic acids of the Krebs Cycle to give the amino acids necessary for the synthesis of proteins and protoplasm. In addition to its synthetic function, for example the synthesis of nucleoproteins, phosphorus is involved in the energy relations of the plant. It is in this way tied to the powering of the anabolic processes of the algae. Black (2) recorded considerable fluctuations in organic nitrogen and a sharp maximum occurred in the early spring when

the nitrate content of the sea water was at a maximum. General agreement was obtained by Macpherson and Young (18). The total nitrogen content of *Macrocystis* and *Nereocystis* was high in the spring, the period of rapid growth and the rapid metabolism essential for solute intake. A minimum in August coincides with the period of minimum nitrate concentration in the waters of San Juan Channel (20). The phosphate content of these algae also parallels that in these waters and in the Strait of Georgia (13) being at a high in winter and a minimum in August.

Fats are storage forms and would be expected to be at a minimum during periods of most active growth and to accumulate in mature plants. Maximum fat content (ether-solubles) was measured in March–April, the period when the collections consisted mainly of the remains of the old plants. The value fell precipitously in May 1950, and in the fronds of *Nereocystis* particularly, began a steady climb to its 1951 maximum in April. In these fronds algin declined steadily from July to April. The reciprocal relationship of fat and algin in these fronds may be significant. The pronounced maximum of mannitol during February–April, the sharp drop in May, and the steady state until the following February, make it difficult to relate mannitol to fat synthesis.

Acknowledgments

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MICROFUNGI

II. *THYROSTROMELLA* HÖHN. AND *THYROSTROMELLA* SYD.[1]

BY S. J. HUGHES[2]

Abstract

Thyrostromella Höhnel (1919) is based on *Sporidesmium myrianum* Desm.; this is illustrated and redescribed. *Thyrostromella* Sydow (1924) is a later homonym; it is based on *Coryneum trimerum* Sacc. which, with four other congeneric North American species, is illustrated and redescribed in *Coryneum*.

Thyrostromella Höhn.

In a short and easily overlooked paragraph, Höhnel (5) published his generic name *Thyrostromella* in 1919 as follows, '*Sporidesmium myrianum* Desm. 1852 = *Macrosporium heterosporium* Desm. 1852 ist eine mit *Thyrostroma* v. H. 1911 verwandte Tuberculariee: *Thyrostromella myriana* (D.) v. H. n. G. (Stroma intraepidermal)'. However, the admittedly short diagnosis, and the single species transferred to the new generic name is sufficient to validate it. In 1929, in a posthumous publication edited by J. Weese, Höhnel (7) redescribed *Thyrostromella myriana* (Desm.) Höhn. from the type collection; he indicated anew how the genus differs from *Thyrostroma* Höhn. but he did not refer to his previous publication of the name in 1919.

Thyrostromella myriana syn. *Spegazzinia ammophilae* Rostr. is redescribed below from the type collections on which these two names are based and from one other collection; the fungus is illustrated in Fig. 1.

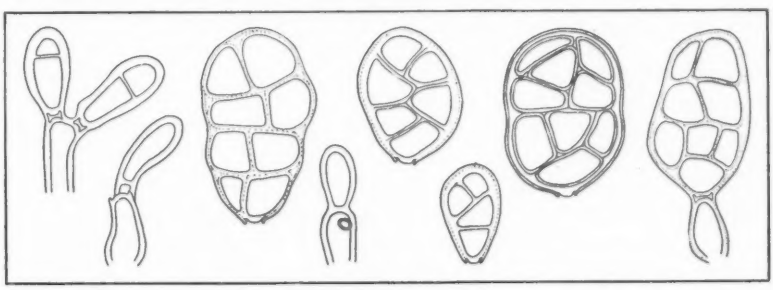


FIG. 1. *Thyrostromella myriana;* conidiophores and conidia from the type collection of *Sporidesmium myrianum;* × 1000.

Thyrostromella myriana (Desm.) Höhnel, Ber. d. deutsch. Botan. Ges. 37 : 157. 1919.

≡ *Sporidesmium myrianum* Desm., Ann. Sci. nat. III, 18 : 357. 1852.

= *Spegazzinia ammophilae* Rostrup, Botan. Tidsskr. 20 : 136. 1896.

Colonies effuse, punctate, causing no discoloration.

[1] *Manuscript received March 15, 1955.*
Contribution No. 1447 from the Botany and Plant Pathology Division, Science Service, Canada Department of Agriculture, Ottawa, Ontario.
[2] *Mycologist.*

Mycelium immersed, composed of hyaline, branched, septate hyphae 1–2 μ wide; here and there, hyphae become aggregated intraepidermally to form small stromata composed of colored cells which are larger than the mycelial hyphae. At maturity the erumpent stromata are each surrounded by a ring of recurved outer epidermal wall giving them a definite margin; they are more or less circular in outline, flattened or slightly raised, black, 25–75, mostly 50–60 μ wide or larger by confluence and then elongated and up to 140 μ long. The stromatic cells are oval or subspherical, 4–6.5 μ in diameter, thick-walled, arranged in more or less regular columns, subhyaline towards the inside and brown to dark brown in the outer layers.

Sporogenous cells form a compact palisade over the entire exposed surface of the stroma; they are oval to obclavate, continuous, thick-walled, brown to dark brown, 10-17 μ long, up to 8 μ wide at the base, tapering slightly to a more or less cylindrical upper part which is 4–6 μ wide, with a terminal scar and occasionally a lateral one as well. The scar is a curious one (Fig. 1), clearly showing the double nature of the wall.

Conidia develop singly as the blown-out apex of the sporogenous cell and of a new growing point when one develops. At first they are subhyaline, fusoid or oval, and continuous, delimited from the sporogenous cell by a transverse wall; then they enlarge, becoming more broadly oval, regularly or irregularly transversely 3-septate with some longitudinal or oblique walls, and brown to dark brown. During maturation a space arises at the apex of the sporogenous cell and in optical section appears as broadly H-shaped; this is often quite dark and opaque as if due to its being filled with air. At this stage the conidium is held on to the sporogenous cell only by means of the continuous outer wall; this wall eventually breaks, the conidium is freed, and the characteristic scar results on sporogenous cell and conidium alike. At maturity, therefore, the dictyospores, which are thick-walled, are constricted at the septa and even waisted at the central transverse septum; they are minutely roughened, vary considerably in size, and measure 21–32 × 13–19.5 μ.

Habitat: On leaves and culms of *Ammophila arenaria*.

Collections examined: (1) The type collection of *Sporidesmium myrianum* in Herb. NY[3], Desmaziere's Pl. Crypt. de France 2, 'En été, sur le chaume et les feuilles sèches du *Calamagrostis arenaria*. Trouvé, par M. Tillette de Clermont, dans les dunes d'Étaples'; (2) The type collection of *Spegazzinia ammophilae* in Herb. C, '*Spegazzinia ammophilae* n. sp. (*Psamma arenaria*) .....vii. 94, Sporodochia 45–80 μ diam. Conid. fusca, 18–26 × 8–16 μ. E.R. [scr. Rostrup]'; (3) Westendorp and Wallays, Herbier Cryptogamique 861, '*Sporidesmium myrianum* [as '*marianum*'] Desmaz. Sur les feuilles et les chaumes sèches de l'*Arundo arenaria*, dans les dunes d'Ostende. Juin' (in Herb. NY).

[3] *The abbreviations for various Herbaria are those given in Index Herbariorum (Regnum Vegetabile, 2 (part 1): 1–179. 1954).*

Grove (4) published a note on the identity of *Sporidesmium myrianum* and *Spegazzinia ammophila*. He pointed out that the fungus is not a true *Spegazzinia* and that it might remain in *Sporidesmium* 'until that genus is subdivided upon modern principles'.

(*Thyrostromella* Syd.)

The earlier and valid *Thyrostromella* Höhn., published in 1919, has been discussed above. In 1924, however, the same generic name was proposed independently by Sydow (apud Sydow and Petrak (11)), for the single [type] species *Coryneum trimerum* Sacc. which Sydow redescribed from the type collection. *Thyrostromella* Sydow was stated to differ from *Thyrostroma* Höhn. in its phragmosporous conidia.

Saccardo (10), Clements and Shear (2), Petrak (9), and Ainsworth and Bisby (1) all ignored *Thyrostromella* Höhn. (1919). Clements and Shear (2) listed *Thyrostromella* Syd. as a synonym of *Coryneum* Nees ex Fr. and also of *Marcosia* Syd. and Syd.[4].

When Hughes (8) made use of the name *Thyrostromella* Syd. in 1953 for three North American fungi he was unaware that the name was illegitimate. These fungi are generically distinct from the type species of *Thyrostromella* Höhn. and a revised disposition is necessary. In Herb. DAOM[4], collections previously assigned to *Thyrostromella* Syd. are now disposed in the *Coryneum* folders.

Had Sydow's generic name not been a later homonym, it would have provided a useful name for the fungi described below. These produce dark brown to black, erumpent, pulvinate or flattened stromata on corticated twigs. On the stromata are produced crowded, cylindrical, oval, or flask-shaped annellophores bearing a succession of thick-walled, pale brown to brown, dry, usually oval chlamydospores which are more or less constantly 2- or 3-septate.

Coryneum trimerum Sacc., Nuovo G. Botan. Ital. 27 : 84. 1920.

≡ *Thyrostromella trimera* (Sacc.) Syd. apud Sydow & Petrak, Ann. Mycol. 22 : 406. 1924.

In his redescription of the type collection Sydow (apud Sydow and Petrak (11)) obtained the following measurements; conidiophores 15–38 × 5–7 μ, conidia 25–45 × 15–18 μ. I have not seen the type collection, from Spokane, Wash., U.S.A., but another (Fig. 2) preserved in Herb. BPI, is undoubtedly *Coryneum trimerum*. This is on the type host, branches of *Robinia pseudacacia*, from Coeur d'Alene, Idaho, U.S.A., v. 1916, coll. J. R. Weir, and determined as this species presumably by the collector.

Annellophores are ovate to subcylindrical, mostly hyaline, sometimes subhyaline, 16–29 × 9.7–13 μ, with one or two annellations.

[4] *Marcosia Sydow and Sydow (12) is based on M. ulei Syd. and Syd., a foliicolous tuberculariaceous hyphomycete on Cynometra bauhiniaefolia from Brazil; this is quite distinct from Thyrostromella Höhn.*

Chlamydospores are ellipsoid 2–(rarely 3–) septate, smooth, not constricted at the septa when young, rounded at the apex, flattened at the basal scar which bears an outer fringe of wall material, pale brown to brown, and measure 32.5–42 × 17.5–21 μ. The cells of old detached chlamydospores may expand considerably, thus appearing strongly constricted; such chlamydospores measure up to 49 μ long and 24 μ wide.

Coryneum negundinis Berk. & Curt. apud Berk., Grevillea, 2 : 153. 1874.

≡ *Exosporium negundinis* (Berk. & Curt.) Höhn., Mitteil. Botan. Inst. Techn. Hochsch. Wien, 6 : 41. 1929.

≡ *Thyrostromella negundinis* (Berk. & Curt.) Hughes, Can. J. Botan. 31 : 619. 1953.

= *Helminthosporium subcuticulare* Ell. & Everh., J. Mycol. 4 : 114. 1888.

= *Coryneum negundinis* Ell. & Everh., Bull. Torrey Botan. Club, 24 : 292. 1897.

≡ *Coryneum septosporioides* Sacc. & Syd. [nom. nov.], in Saccardo's Sylloge Fungorum, 14 : 1022. 1899.

Annellophores are oval to subcylindrical and tapered towards the apex, or flask-shaped, continuous or basally 1-septate, hyaline to subhyaline, 11–30 × 6–9.5 μ with up to four close annellations.

Chlamydospores are ellipsoid, pale brown to brown, thick-walled, 2–(rarely 3–) septate, smooth, not at all or scarcely constricted at the septa, somewhat flattened at the basal scar, 3.2–6.4 (mostly 4–5 μ) in diameter, which bears a minute circular frill; they measure 27–39 × 13–18 μ.

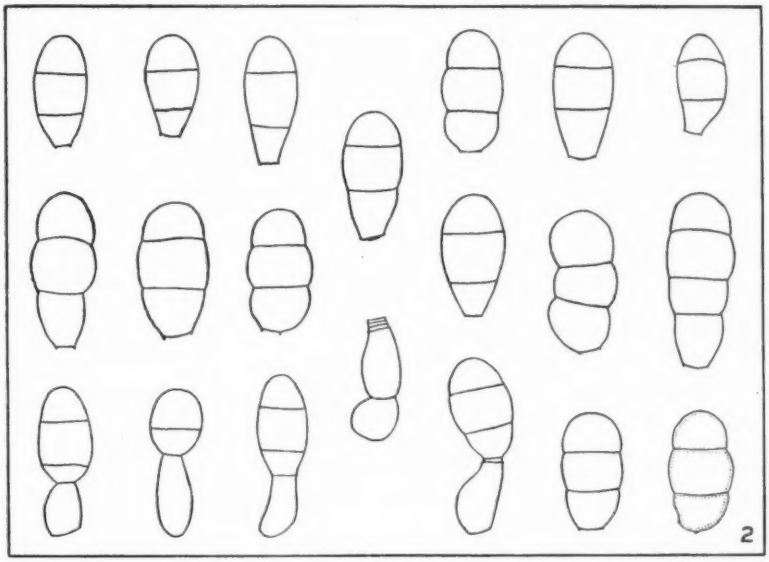
Habitat: On twigs of *Acer negundo* (*Negundo aceroides*).

Collections examined: (1) The type collection of *Helminthosporium subcuticulare* in Herb. NYS, Parasitic Fungi of Montana No. 169, Sand Coulee, Mont., U.S.A., 10.iv.1888 (Fig. 3); (2) The type collection of *Coryneum negundinis* Ell. and Everh., Fungi Columbiani 1161, Denver, Colo., U.S.A., iii.1897 (DAOM 36001); (3) DAOM 36003, Fort Collins, Colo., iii. 1898, det. J. B. Ellis as *C. negundinis* Berk. and Curt.; (4) DAOM 3447, Edmonton, Alta., Canada, 30.ix.1933; (5) DAOM 12128, Vesper, Sask., Canada, 1942; (6) '*Coryneum negundinis* B. & C. on *Negundo aceroides*, Denver, Colo., Feb. 1897 (Bethel, 195) [scripsit J. B. Ellis]' in Herb. NY.

I have not seen the type collection of *Coryneum negundinis* Berk. and Curt., but Dr. R. W. G. Dennis (in lit.) kindly illustrated the fungus for me from the type in Herb. K and stated that the conidia measure 26–33 × 13–16 μ. Berkeley and Curtis described their species from North Carolina on *Acer negundo*.

Dearness (3) recorded *Coryneum septosporioides* (= *C. negundinis*) on still living branches of *Acer negundo* from Wyoming and Saskatchewan: he stated that the 'fungus can produce a serious canker on branches' of this tree.

Höhnel (6) included *Coryneum vogelianum* Sacc. and *C. negundinis* Berk. & Curt. in *Exosporium* and considered *C. negundinis* 'Ell. & Everh.' to be only a form of the first named but with larger and dark colored spores. The type



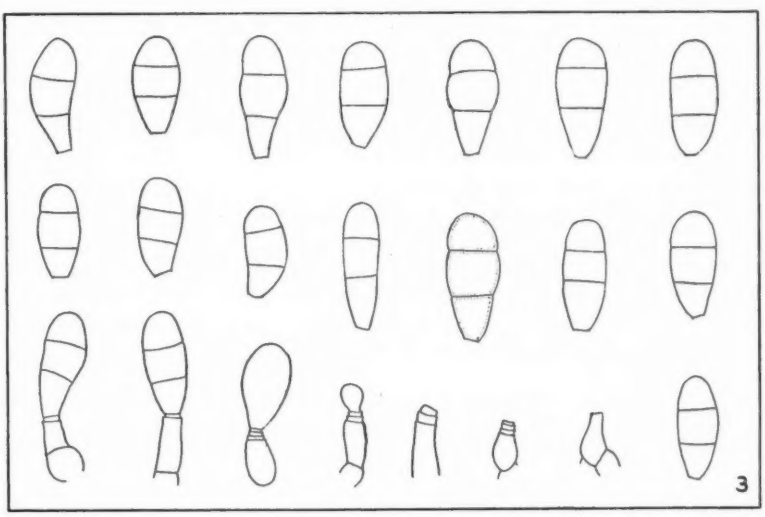


FIG. 2. *Coryneum trimerum;* annellophores and chlamydospores; × 500.
FIG. 3. *Coryneum negundinis;* annellophores and chlamydospores from the type collection of *Helminthosporium subcuticulare;* × 500.

collection of *Coryneum vogelianum* (Sydow, Mycotheca germanica 437, on *Acer campestre*, Germany, 24.5.1905, leg. P. Vogel, in Herb. PAD) shows smaller, subhyaline to very pale brown 2-septate chlamydospores developing from crowded annellophores which are up to 50μ long. The fungus is certainly congeneric with *Coryneum negundinis* and not an *Exosporium*.

Coryneum pedunculatum (Ell. & Everh.) comb. nov.

≡ *Brachysporium pedunculatum* Ell. & Everh., Proc. Acad. Nat. Sci. Phil. 47 : 440. 1895.

≡ *Exosporium pedunculatum* (Ell. & Everh.) W. B. Cooke, Mycologia, 41 : 617. 1950.

≡ *Thyrostromella pedunculata* (Ell. & Everh.) Hughes, Can. J. Botan. 31 : 619. 1953.

This fungus was illustrated by Hughes (8).

Annellophores are subcylindrical to flask-shaped, continuous or basally 1-septate, subhyaline to pale brown, 16–32 × 9.7–13.5μ with up to six conspicuous annellations.

Chlamydospores are ellipsoid, brown, thick-walled, 2– (rarely 3–) septate, smooth, slightly constricted, flattened at the basal scar which is 4–10μ in diameter and which has a minute circular frill; they measure 24–39 × 12–15μ.

Habitat: On twigs of *Sambucus* spp.

Collections examined: (1) The type collection of *Brachysporium pedunculatum* in Herb. NY, on *Sambucus glauca*, Pullman, Wash., U.S.A., 24.iii.1894, C. V. Piper 316, labelled *Brachysporium puccinioides* [nomen nudum]; (2) W. B. Cooke, Mycobiota of North America 208, sub *Exosporium sambuci* Tracy and Earle, on *Sambucus glauca*, Mt. Shasta, Calif., U.S.A., 25.vii.1946, det. Lee Bonar; (3) DAOM 1749, on *S. glauca*, near Northport, Wash., 24.viii.1929; (4) Herb. BPI 52731(F.P.), on *S. glauca*, near Northport, Wash., 26.viii.1929; (5) Herb. BPI 52702(F. P.), on *S. glauca*, Northport, Wash., 20.viii.1929; (6) Herb. NY, on *S. glauca*, Spokane Co., Wash., 5.viii.1949, W. B. & V. G. Cooke (25335) sub *Exosporium pedunculatum;* (7) Herb. NY, on *Sambucus glauca*, Revelstoke, B.C., Canada, J. R. Hansbrough (158), 25.ix.1930 sub *Exosporium sambuci;* (8) Herb. BPI 52727(F. P.), on *Sambucus melanocarpa*, Leadpoint, Wash., 24.viii.1929; (9) Herb. BPI 52728(F. P.), on *S. melanocarpa*, Northport, Wash., 26.viii.1929.

Coryneum sambucinum Ell. & Everh., Bull. Torrey Botan. Club, 24 : 467. 1897.

≡ *Thyrostromella sambucinum* (Ell. & Everh.) Hughes, Can. J. Botan. 31 : 619. 1953.

= *Exosporium sambuci* Tracy & Earle, Plantae Bakerianae, 1 : 36. 1901.

This fungus was illustrated by Hughes (8).

Annellophores are more or less cylindrical, continuous or 1-septate, up to 65μ long and 8μ wide but generally shorter; they are hyaline to subhyaline and bear up to six close annellations.

Chlamydospores are ellipsoid, 3- (rarely 2- or 5-) septate, smooth, brown to dark brown, constricted at the septa at maturity, and flattened at the basal scar which has a minute circular frill; they measure 35–55 (66) × 15–27 μ.

Habitat: On branches of *Sambucus* spp.

Collections examined: (1) The type collection of *Coryneum sambucinum* in Herb. NY, on *Sambucus*, Buena Vista [Colo., U.S.A.], vi.1897, Bethel (315a); (2) The type collection of *Exosporium sambuci* in Herb. BPI, Plants of Southern Colorado 1104, on *Sambucus melanocarpa*, Upper La Plata River, 13.vii.1898; (3) Crypt.-Format. Coloradensium 68, sub *Brachysporium sphaerocolum* n. sp. [nomen nudum], on *Sambucus microbotrys*, Larkspur Dell, 11.vii.1905, in Herb. BPI; (4) Herb. BPI 65365, on *Sambucus*, Nevada [U.S.A.], 28.vi.1924, W. A. Archer; (5) on *S. glauca*, San Antonio Canyon, near Claremont, Calif., C. F. Baker, 10.x.1903, det. [J. B.] Ellis as *Brachysporium pedunculatum*, in Herb. BPI; (6) on *Sambucus canadensis*, Artists Glen [? Calif.], 1924, Lee Bonar, in Herb. BPI.

Coryneum rhoinum (Dearn. & Barth.) comb. nov.

≡ *Exosporium rhoina* Dearn. & Barth., Mycologia, 21 : 332. 1929.

Annellophores are ovate, cylindrical or flask-shaped, continuous, hyaline to subhyaline, 10–23.5 × 5.8–16.5 μ, with up to three annellations.

Chlamydospores are ellipsoid to clavate, 2-septate, smooth, very slightly if at all constricted at the septa, rounded at the apex, flattened at the basal scar which is 4.8–6.4 μ in diameter and which has a minute circular frill; they measure 35.5–55 × 15.5–19.5 μ.

Habitat: On branches of *Rhus glabra*.

Collection examined: On branches of 1952 growth of *Rhus glabra*, Lavington, B.C., Canada, 27.iv.1953, leg. J. Grant, DAOM 35484 (Fig. 4). The collector

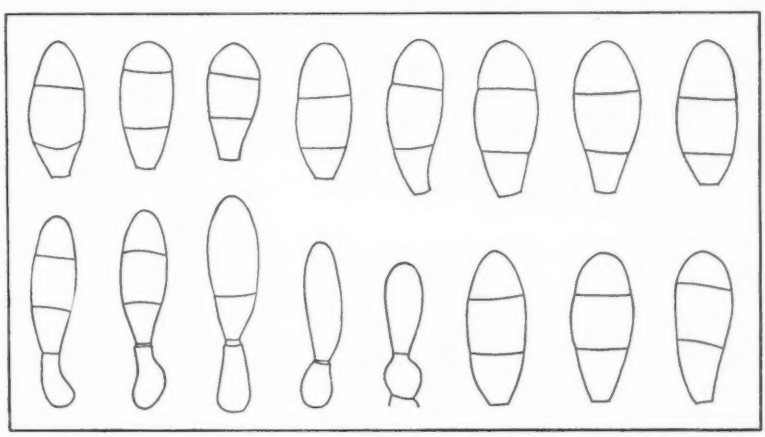


FIG. 4. *Coryneum rhoinum;* annellophores and chlamydospores from DAOM 35484; × 500.

stated that the fungus was associated with death of shoots starting at ground-line.

I have not seen the type collection which was described on *Rhus glabra* from Moscow, Idaho, U.S.A., June, 1917. The 3-septate conidia were described as 28–42 × 13–19μ.

In the second section of this paper (*Thyrostromella* Syd.) are included five taxa which are superficially and microscopically very similar. One of these, *Coryneum sambucinum*, is readily distinguished by its 3-septate chlamydospores. The others produce 2-septate chlamydospores and their separation into four taxa is based far more easily on the identity of the host than on the slight differences in morphology. A greater number of collections on the various hosts is necessary in order to appraise such differences. It is quite possible that the four taxa represent only one species; cultures of the fungi from the different hosts, *Robinia*, *Acer negundo*, *Sambucus*, and *Rhus glabra* might well solve the problem. In view of the uncertainty the four available epithets are used.

All but one of the collections cited above and assigned to the four names were made in northwestern United States and western Canada (Wash., Idaho, Mont., Calif., Colo., and B.C., Alta., Sask.); the exception is the type of *Coryneum negundinis* Berk. & Curt. which was collected in North Carolina. Dearness (3) recorded one species from Wyoming, U.S.A.

Other species assigned to *Thyrostromella*

T. eximia (Sacc.) Petrak, Ann. Mycol. 23 : 143. 1925.

≡ *Coryneum eximium* Sacc., Ann. Mycol. 12 : 294. 1914. On twigs of *Carpinus betulus;* Moravia.

T. foliicola Petr. & Ciferri, Ann. Mycol. 30 : 351. 1932.

On living leaves of *Lonchocarpus latifolia;* Dominican Republic.

T. hartigiana (Sacc.) Petr., Ann. Mycol. 32 : 430. 1934.

≡ '*Septogloeum hartigianum* Sacc. apud Hartig, R., Forstl.-Naturwiss. Zeitschr. 1 : 289. 1892. On branches of *Acer campestre;* Bavaria'.

≡ *Exosporium hartigianum* (Sacc.) Höhn., Mitt. botan. Inst. Techn. Hochsch. Wien, 6 : 41. 1929.

T. salicella Moesz & Smarods apud Moesz, 'Bot. Közlem, 35 : 56. 1938. On *Salix cinerea*, Latvia.'

In *T. eximia* and *T. foliicola* the spores were described with many transverse septa.

Saccardo described the spores of *Septogloeum hartigianum* as 2-septate and 24–36 × 10–12μ; but Petrak based his transference of this species to *Thyrostromella* on the examination of a collection with spores 14–23 × 6.5–10μ. Petrak's measurements are close to those of the 2-septate chlamydospores of *Coryneum vogelianum* Sacc. viz. 16–20 × 8μ (see p. 344). However, the first three species are undoubtedly misplaced in *Thyrostromella* Höhn. I have not seen a description of *T. salicella.*

For the loan of collections in their keeping I am much obliged to Dr. N. Fabritius Buchwald, Professor C. Cappelletti, Dr. R. G. W. Dennis, Dr. D. P. Rogers, Mr. S. J. Smith, and Mr. J. Stevenson. My thanks also are due to Dr. G. R. Bisby and Dr. D. P. Rogers for assistance with the literature.

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DOWNY MILDEW OF *DACTYLIS GLOMERATA* CAUSED BY *SCLEROPHTHORA CRYOPHILA*[1]

By Walter Jones[2]

Abstract

A disease of orchard grass (*Dactylis glomerata* L.) caused by a downy mildew, *Sclerophthora cryophila* n. sp., was first found in Saanichton, near Victoria, B.C., in 1948. The symptoms appear on the leaves as light brown, oval-elongate to irregular areas, and as interveinal and marginal streaks. The inflorescences are occasionally affected. Zoospores are liberated from sporangia at 1° C., but the most favorable temperature for their emergence is between 4° and 9° C. The disease is considered of economic importance, particularly where orchard grass is grown for seed. Control involves the use of seed from disease-free crops and the application of fungicidal sprays or dusts during late autumn and late winter.

Introduction

A downy mildew of orchard grass, *Dactylis glomerata* L., was first found by Jones (1) in June, 1948, in the strain test rows at Saanichton, near Victoria, B.C. The grazing potential of two early strains of the grass was reduced materially by the disease. In general appearance, the symptoms on the leaves were somewhat similar to those associated with frost injury. During that year, the precipitation in spring and early summer was above normal, and these conditions appeared to favor the development of the disease.

When the diseased leaves were examined, numerous clusters of *Phytophthora*-like sporangia were present on the stomata of the ventral surfaces (Fig. 1) and *Sclerospora*-like organs of fusion were found within the mesophyll tissues (Fig. 10).

In 1949, sporangia of the fungus were observed on diseased leaves on April 14, when the minimum temperature was 45° F. and the maximum 58.5° F. In 1950, the fungus was sporulating on diseased leaves on August 30, when the minimum and maximum temperatures were 49° F. and 66° F. respectively, and it appeared that the sporangia had developed during the night when minimum temperatures prevailed. On February 22, 1954, the disease was again prevalent in three plots and in adjacent fields, and sporangia were present in abundance on affected leaves collected that day. The minimum and maximum temperatures recorded above ground on that day were 39.5° F. and 46° F. respectively, and it was evident from field observations that the fungus was pathogenic at these relatively low temperatures. The large number of sporangia on the leaves also indicated that the fungus had been sporulating for a considerable length of time. In November, 1954, the disease was found on orchard grass grown for ensilage in a field in the lower mainland of British Columbia.

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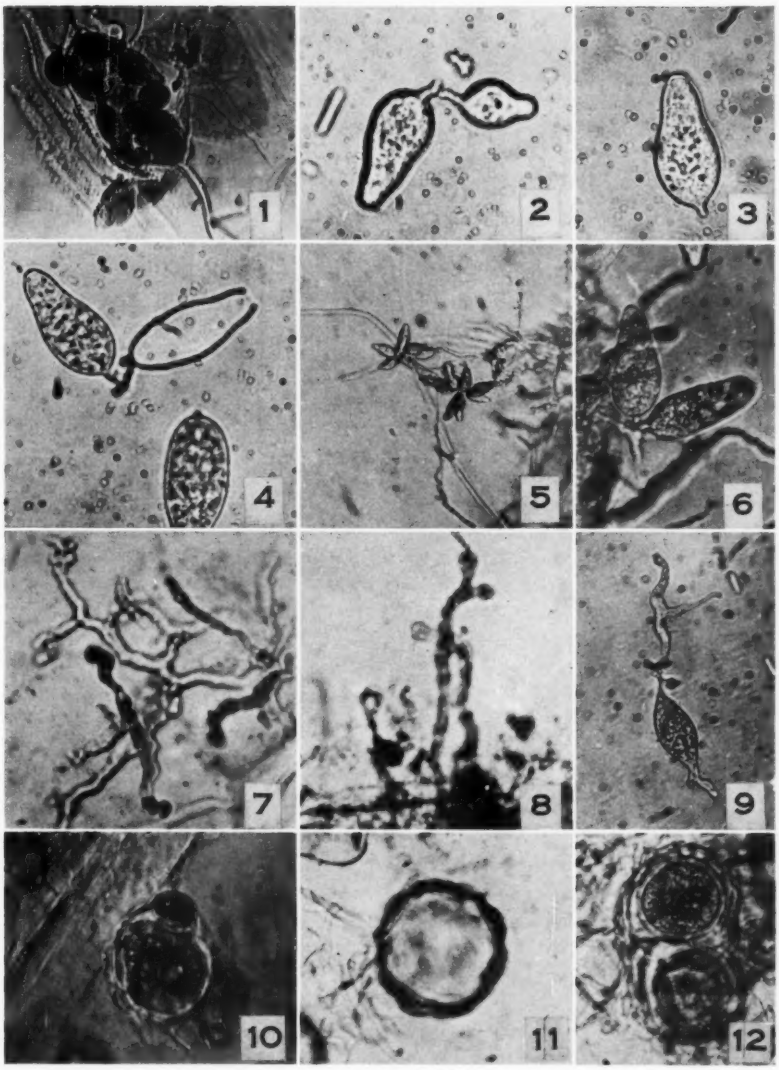

FIG. 1. Numerous sporangia closely adjacent to a stoma. FIG. 2. Sporangia. FIG. 3. Mature sporangium with persistent pedicel. FIG. 4. Showing a typical empty sporangium after emergence of zoospores. FIG. 5. Sympodial clusters of sporangia which developed from mycelium growing from diseased tissue in water. L.P. FIG. 6. As above, in water, showing the close sympodial branching, one sporangium being terminal and the other lateral. H.P. FIG. 7. Showing the character of mycelium growing from diseased tissue in water. FIG. 8. A sterigma-like sporangiophore atop a hypha, from diseased tissue in water. FIG. 9. Germinating sporangium. FIG. 10. Organs of fusion—paragynous antheridium and an oogonium. FIG. 11. Oogonium, showing the sinuous wall. FIG. 12. Oospores in mesophyll.

The characteristic morphological features of the fungus were the *Phytophthora*-like sporangia and the *Sclerospora*-like organs of fusion. These were similar to those of the genus *Sclerophthora*, described in 1953 by Thirumalachar *et al.* (5), but the sporangia and organs of fusion of the fungus on orchard grass were significantly smaller than those of the designated type species, *Sclerophthora macrospora* (Sacc.) Thirumalachar *et al.* The characteristic physiological feature of the fungus was its ability to produce sporangia at low temperatures.

It is considered that the fungus is an undescribed species of the genus *Sclerophthora* and, consequently, the symptoms of the disease, the influence of temperature on the emergence of zoospores from sporangia, and a description of the fungus are presented in this paper.

Symptoms of the Disease

On the leaf blades, the symptoms appear as light brown, oval-elongate to irregular areas, and as interveinal and marginal streaks. The surrounding areas are often yellowish in color. Sporangia are usually present on the ventral surfaces of affected areas. The inflorescences are occasionally infected. The symptoms on these appear as pale-cream to pale-brown discoloration of the sheaths of the stems and of the spikelets. Distortion of the floral parts has also been observed in a few cases. Root- and crown-rot symptoms have not been seen, but sporangia are often present on the sheaths of the basal parts of affected plants.

The most dependable diagnostic symptom in the field is the presence of whitish clusters of sporangia closely adjacent to the stomata of the ventral surfaces of the diseased areas of the leaves. The sporangia are best seen with a hand lens. At first, these appear whitish but later become somewhat creamy. When diseased tissues bearing sporangia are further examined microscopically, organs of fusion are invariably present among the mesophyll cells, and their prevalence is another reliable diagnostic feature.

Since sporangia develop readily at relatively low temperatures, the symptoms are more easily detected on the new leaves in late winter and early spring before they become infected by other leaf-spotting fungi that often cause somewhat similar symptoms. Among the latter, the most confusing symptom is that of brown stripe caused by *Scolecotrichum graminis* Fckl.

Emergence of Zoospores from Sporangia at Different Temperatures

Since sporangia of the fungus were found on diseased leaves in the field as early as February 22, when the temperatures were relatively low, an experiment was conducted to determine the effect of different temperatures on the discharge of zoospores from sporangia. For this purpose, small sections cut from diseased leaves bearing sporangia were inserted in syracuse dishes containing distilled water. These dishes were then placed in incubators maintained at different temperatures.

In a preliminary series, it was found that after 18 hr., no zoospores had emerged at −6°, −4°, −2°, and 24° C., but at 1° C. numerous empty sporangia and motile zoospores were observed. In three other series, where the temperatures in the incubators were set at 1°, 4°, 9°, 13°, 17°, and 21° C., and observations made after 18 hr., it was found that 4° and 9° C. were the most favorable temperatures for the discharge of the zoospores. Many had emerged at 1°, 13°, and 17° C., but only a few were seen in the 21° C. series. At temperatures of 1°, 4°, and 9° C., the zoospores were very active, while at 13°, 17°, and 21° C., their movement was slow. At these latter temperatures, a few of the sporangia had germinated directly (Fig. 9).

The results indicate that the approximate minimum, optimum, and maximum temperatures at which zoospores are liberated from sporangia are 1° C., 4–9° C., and 21° C. respectively.

The Fungus

The only species described in the genus *Sclerophthora* is the type species *Sclerophthora macrospora* (Sacc.) Thirumalachar *et al.* syn. *Sclerospora macrospora* Sacc., *Phytophthora macrospora* (Sacc.) Tanaka.

The dimensions of the sporangia and organs of fusion of this species given by Thirumalachar *et al.* (5), Thirumalachar and Narasimhan (6), and Tanaka (4) are significantly larger than those of the *Sclerophthora* sp. found on orchard grass. Thus Tanaka (4) states that the average dimensions of the sporangia of *Phytopthora macrospora* found on wheat in Japan were $74.6 \times 46.8\mu$, and those of the oospores $60.25 \times 56.67\mu$, while from the United States, Miles and Epps (2) state that the average diameter of oospores of *Sclerospora macrospora* found on oats in Mississippi was 55.6μ. In contrast, the average dimensions of the sporangia and oospores of the species on orchard grass are $30.5–38 \times 15–19\mu$ and 31.8μ respectively.

A characteristic physiological feature of the fungus is its ability to produce sporangia at the relatively low temperature of approximately 40° F. during the winter. It thus differs from *S. macrospora* since Thirumalachar *et al.* (5) state that sporangia of the latter species were found to develop whenever infected tissue was incubated at high humidities and at a temperature between 60° and 68° F.

The morphological and physiological characters of the fungus are thus considered to be different from those of the type species, and the downy-mildew disease caused by it has not been previously reported on any member of the tribe Festuceae. Consequently, the binomial *Sclerophthora cryophila* is proposed for the new species.

Sclerophthora cryophila sp. nov.

Sporangiophori breves, sterigmatibus similes, ex hyphis endophyticis orientes, stomatibus protrudentes, sporangia in sympodio monochasiali iis Phytophthorae simila ferentes.

Sporangia numerosa, hyalina, apice perforata, obpyriformia, 22.5–45.5μ, plerumque 30.5–38μ longa, 11.5–22.5μ, plerumque 15–19μ lata, pediculis persistentibus.

Oogonia in mesophyllo posita, numerosa, subglobosa, usque globosa, sinuosa, aurea usque sucineo-fulva, per medium 29.5–51.5μ, plerumque 38.5μ, parietibus 1.9–3.8μ, plerumque 3.7μ crassis.

Antheridia paragynoecea. Oosporae globosae, per medium 20–37.5μ, plerumque 31.8μ, parietibus 1.5–3.5μ, plerumque 2.6μ crassis, cum pariete oogoniali confluentibus.

Sclerophthora cryophila n. sp.

Sporangiophores short, sterigma-like, arising from endophytic hyphae, protruding through the stomata, and bearing *Phytophthora*-like sporangia in a close monochasial sympodium.

Sporangia numerous, hyaline, apically poroid, obpyriform. Length 22.5–45.5μ, average 30.5–38μ, width 11.5–22.5μ, average 15–19μ. Pedicels persistent.

Oogonia in mesophyll, numerous, subglobose to spherical, sinuous, golden to amber-brown, diam. 29.5–51.5μ, average 38.5μ, wall 1.9–3.8μ, average 3.7μ thick.

Antheridia paragynous. Oospores spherical, diam. 20–37.5μ, average 31.8μ, wall 1.5–3.5μ, average 2.6μ thick, confluent with oogonial wall.

Habitat: On living leaves of *Dactylis glomerata* L. coll. Saanichton, B.C., June 1, 1948. Type deposited in the herbarium of the Plant Pathology Laboratory, Saanichton, B.C.

Discussion of Economic Importance and Control Measures

Since the disease was found in the field-test plots in Saanichton in 1948, it has spread to adjacent fields and, in November, 1954, it was found in a Fraser Valley district in the lower mainland of the province. This indicates that the disease may be widely distributed.

The seed of the different strains sown in Saanichton was brought in from Eastern Canada in 1946, but the original source is not known. The seed sown in the Fraser Valley was of European origin.

It is possible that the fungus was introduced with the seed in the form of resting oospores, since these organs are invariably present in the tissues of diseased leaves and inflorescences.

It may be difficult to assay the losses incurred by a disease of this nature, but in 1948 the grazing potential of two early strains of the grass was considered to have been materially reduced in Saanichton. This would seem natural, since the fungus can attack plants during the winter, and the leaves of the early-growing strains would thus be subject to infection at an earlier period than those of the later strains. In the Fraser Valley field, where the disease was found, the grower claimed that the ensilage crop during the season was significantly below average.

Numerous species of *Sclerospora* that cause considerable damage to graminicolous hosts have been reported from different countries, and the species *Sclerophthora macrospora* is also widely distributed. In 1952, Summers (3) claimed that the downy mildew caused by *S. macrospora* caused a loss of 200,000 bushels of oats in Mississippi, and that the incidence was greatest in fertile soil with high moisture content. He also stated that affected plants in many cases were so distorted that they were often mistaken for weed grasses, and that the seed oats should be obtained from fields known to be free of the disease. It therefore appears that the disease of orchard grass caused by the downy mildew *Sclerophthora cryophila* should be considered of potential economic importance, particularly in districts where the grass is grown for seed.

One obvious method of control is to obtain seed from disease-free crops, since it has been shown that oospores of the causal fungus are readily produced in diseased tissues, and the oospores could easily be carried in diseased tissue fragments along with the seed. Where the disease is already present in pasture crops, it should be possible to reduce infection by applying fungicidal sprays or dusts. Because the temperature for growth of the fungus is comparatively low, it is considered that the best time for application is in late autumn and late winter.

Acknowledgments

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THE REACTIONS OF CERTAIN SOLANACEOUS SPECIES TO STRAINS OF POTATO VIRUS Y[1]

By James Munro[2]

Abstract

Strains of potato virus Y that were not clearly differentiated from each other by their reaction on potato varieties were shown to be separate entities by the differing reactions that they caused in several other solanaceous species. One strain did not infect *Physalis floridana*, but this same strain caused necrosis and a severe distortion of the young leaves of *Nicotiana sanderae*. After infection with certain other strains, *N. rustica* showed leaf-drop streak symptoms; this observation has not been previously reported. An avirulent strain in all potato varieties and seedlings tested so far caused a severely necrotic disease in *N. tabacum* and other *Nicotiana* species.

Introduction

Bawden and Kassanis (1) have demonstrated that potato virus Y occurs in strains that cause widely differing symptoms in potato varieties. Except in the studies of Hutton and Peak (4), differentials for strains of this virus have not been sought in other solanaceous species, which have, rather, been used as local lesion hosts and plant indicators of the virus.

During the course of studies on strains of potato virus Y by the author, solanaceous plants other than the potato were also used, and the purpose of this paper is to describe some of the departures from the common symptom pictures that resulted from reactions between strains of this virus and some of these hosts.

Materials and Methods

The strains of virus Y selected are referred to as Y1, Y2, Y3, Y4, Y5, Y6, Y7, and V. Nec. They bear no relation to any similarly named Y strains in the work of Bawden and Kassanis (1, 2) except that V. Nec., an abbreviation for "veinal necrosis", is probably the same strain that they described as "tobacco veinal necrosis virus". The first seven are described and received the numeral in the order in which they were found or obtained.

Potato plants were examined in field and garden and a selection was made of those which showed mosaic and necrotic symptoms in degrees of severity not commonly associated with virus Y in those varieties. Leaf samples were taken; the sap was expressed; and inoculations were made to *Datura stramonium* L. and to *Nicotiana tabacum* L. var. White Burley, the plant indicators commonly used for viruses X and Y. Where both viruses were found to be in the tobacco plants inoculated from any one sample, transmissions were also made by the aphid *Myzus persicae* Sulz. to additional tobacco seedlings. In these ways isolates Y1, Y4, Y5, Y6, and Y7 were obtained.

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Some of this work is part of a thesis submitted to the University of Durham in partial fulfillment of the requirements for the Master of Science degree, October, 1950.

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Strains Y2 and Y3 were obtained from Dr. W. R. S. Wortley of the Commonwealth Potato Station, Cambridge, England. All the foregoing strains were mutually antagonistic, that is, all plants systemically infected by any one resisted invasion by any other. The eighth strain, V. Nec., was made available by Dr. Kenneth M. Smith, Director of the Plant Virus Research Station, Cambridge, where some of this work was carried out. This virus strain had been isolated from tubers brought to Cambridge in the Commonwealth Potato Collection from South America. V. Nec. was identified by Nobrega at Cambridge as being similar to, or identical with, the virus isolated by Nobrega and Silberschmidt (5) that causes "Necrose das Nervuras" of tobacco (*Nicotiana tabacum*, var. White Burley). They considered it to be a strain of potato virus Y.

The virus strains were maintained in young plants of White Burley tobacco. Inoculations to the host plants to be described were made by rubbing their leaves with the forefinger wet with infective sap from macerated tobacco leaves. The inoculations were made after the leaves of the plants had been dusted with 400-mesh carborundum to facilitate transmission.

Symptomatology

Lycium halimifolium and L. rhombifolium

Dennis (3) described the use of *Lycium barbarum* as a new test plant for potato virus Y and observed that on this plant the virus caused numerous brown local lesions to develop on the leaves. These leaves then became chlorotic and dropped.

In 1947 it was learned from the Curator of the Cambridge Botanic Gardens that the term *barbarum* may have been used to denote a group of *Lycium* species. The species *Lycium halimifolium* Mill., *L. rhombifolium* Moench., and *L. chinense* Mill. were then obtained from the Botanic Gardens.

In experiments with *L. halimifolium* and *L. rhombifolium* young seedlings or young shoots bearing about 12 to 18 leaves were used. Both species reacted to all the potato virus Y strains, including V. Nec., in the manner described by Dennis. The lesions developed more quickly and were most numerous on the lower and middle leaves of these young plants, but no lesions were observed on the young upper leaves. On *L. chinense*, however, these strains did not cause local lesions. The type and development of the local lesions on *L. halimifolium* and *L. rhombifolium* were not markedly different either between the species with the same strain, or among the strains within a species, nor could comparative differences be observed by the profusion or sparsity of the lesions caused by any of the strains.

Physalis floridana

Ross (6) found that *Physalis floridana* Rydb. proved to be a good local lesion test plant under most conditions. However, its greatest value appears to be in differentiating strains of virus Y on the basis of the speed of the reaction and the degree of severity of foliar necrosis (4).

The plants of *P. floridana* were inoculated when they were about three inches high. Within eight days necrotic lesions developed on the inoculated leaves of all plants except those inoculated with strain Y3 (Fig. 1). Symptoms of the same general type were caused by each of the remaining strains; chlorotic lesions began at the leaf tips of the oldest uninoculated leaf, and appeared successively towards the leaf base. After the lesions had developed away from the tip of the first leaf, they appeared in the succeeding leaf and progressed up the stem from leaf to leaf towards the growing point. The chlorotic lesions then became necrotic, and joined together to form a necrotic area, followed shortly by abscission. The degree of severity was adjudged by the speed of development of the chlorotic and necrotic lesions within a leaf and from leaf to leaf, and abscission, together with the consequent dwarfing of the plant and the reduced leaf size.

The varying degrees of severity in symptoms caused by the host reaction to the different strains did not bear direct relation to the trend in severity of these same strains in potato varieties. The symptoms induced by the eight strains when inoculated to *P. floridana*, in fact, showed an almost complete reversal of the apparent lethal properties of some strains in potato varieties. The strains Y1 and V. Nec., which cause very mild symptoms in the potato varieties, were the most virulent strains in *P. floridana*. The most severe strain in potato varieties, Y7, was the mildest of the eight strains in this *Physalis* species. Of the five strains that induced mild symptoms in most of the potato varieties, Y1, Y2, Y3, Y4, and V. Nec., the first two and V. Nec., dwarfed and distorted the *P. floridana* plants within 10 days, and V. Nec. and Y1 completely destroyed them within 21 days. But despite repeated attempts it was not possible to infect series of *P. floridana* plants with Y3 either by rubbing inoculation or by using aphids. There was either resistance to infection, or the multiplication of the virus in the host was so limited that the amounts in the sap were insufficient to infect tobacco plants.

Finally, three series of *P. floridana* plants with six plants in each series were inoculated with Y3. After 15 days sap from the plants of series 1 was inoculated to six tobacco plants to test the *P. floridana* for infection. The six *P. floridana* plants in the series were then reinoculated with Y3. Sap from the plants in series 2 was also inoculated to six tobacco plants to check for infection within the series, and the six *P. floridana* plants were then inoculated with strain Y7. Likewise, sap from the plants in series 3 was inoculated to six tobacco plants but these *P. floridana* plants were retained without further treatment. The tobacco plants did not become infected from any one of the series of Y3-inoculated *P. floridana* plants. After another 15 days, series 1 and 3 were again checked for virus Y infection by inoculation to tobacco plants; these tests were also negative. The plants of series 2 showed symptoms typical of infection by Y7 alone. This indicated that if Y3 was present, it was in a concentration too low to prevent the systemic invasion of another strain of the same virus, and for all practical purposes *P. floridana* was immune to Y3.

Nicotiana rustica

Because the plants of *Nicotiana rustica* L. were genetically of many different types, the symptoms that followed inoculation with these strains of virus Y were not considered to be specific virus strain–host reactions. Of the eight strains that were used in repeated tests, four caused an "acropetal necrosis" in at least two or three plants out of each six infected, typical of the leaf-drop streak symptoms of virus Y in many potato varieties (Fig. 2). As far as can be ascertained there is no record of a common field strain of potato virus Y causing leaf-drop streak symptoms in a species of *Nicotiana*. The disease caused in *N. rustica*, like that in potato, is a necrosis of the collenchyma of the plant stem and of the leaf petioles.

Again there was a difference in the necrotic reactions of this species to the strains as compared to the reactions caused by these strains in potato varieties. Three of the mildest potato strains Y1, Y3, and V. Nec., and the most severe potato strain Y7, did not cause acropetal necrosis in any plants of *N. rustica*. But the mild potato strain Y4 was most consistent in causing leaf-drop streak in the majority of the *N. rustica* plants inoculated with this strain.

Nicotiana sanderae

Except for symptoms of vein-clearing followed by vein-banding, there was a normal development of the plants of *Nicotiana sanderae* Sander following inoculations by strains Y1, Y4, Y6, and Y7.

The Y3-inoculated plants did not produce the normal long single main stems; instead, the leaves remained close to the bases of the plants. The young leaves were twisted, distorted, and much reduced in size; and the veins formed a necrotic network (Fig. 3). The older leaves, though not deformed, developed numerous necrotic lesions.

The plants infected with Y2 were dwarfed; but an abnormal profusion of leaves grew out from the center of the plants. There was neither twisting nor veinal necrosis of the young leaves, but there were some necrotic spots on the old leaves.

Plants inoculated with Y5 also remained dwarfed, but the smaller number of leaves than is normal to this species gave an open appearance to the plants. The leaves were elongated and pale in color, with a fine necrosis of the veins on all the leaves.

The symptoms caused by V. Nec. as described on White Burley, were also typical of those that developed on *N. sanderae*.

Nicotiana tabacum var. White Burley

All the strains except V. Nec. caused the same general type of symptoms on tobacco: a vein-clearing within seven to eight days followed by vein-banding in 11 or 12 days. No differences in reactions between strains could be detected in any of the plants.

Bawden and Kassanis (2) found that the "tobacco veinal necrosis virus" was serologically related to other strains of virus Y but that it did not protect

PLATE 1

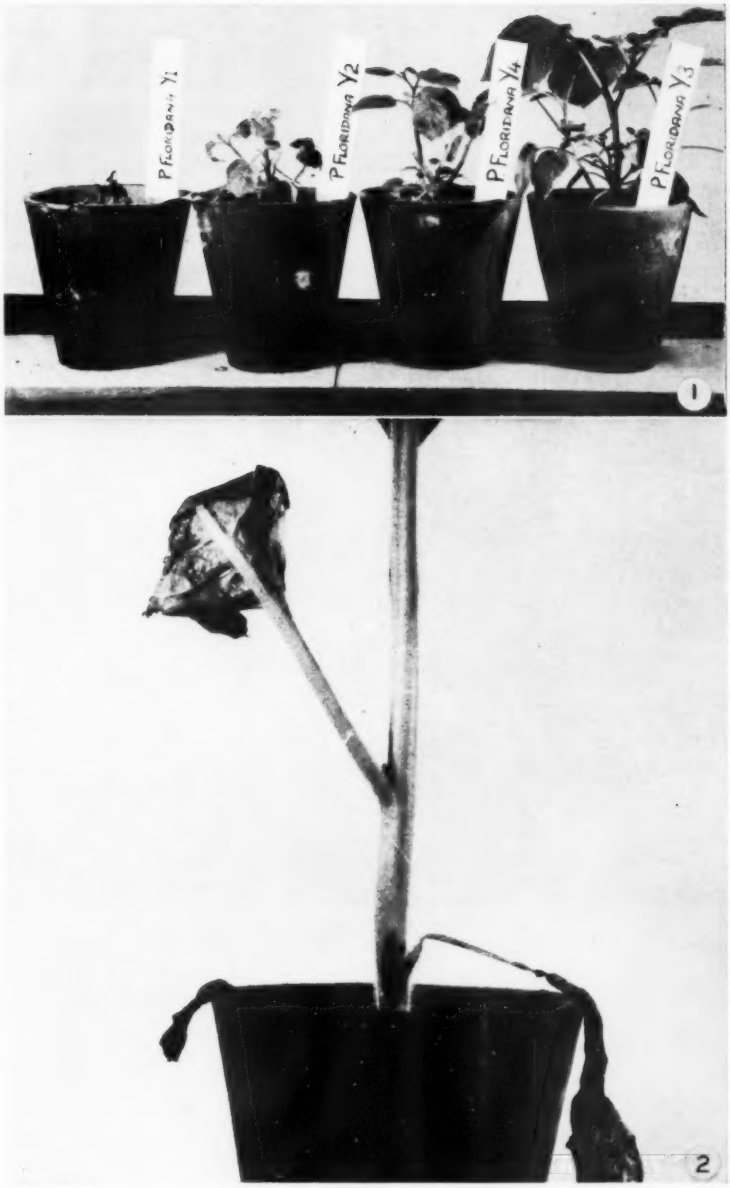

FIG. 1. The effects of four strains of potato virus Y on *P. floridana*.
FIG. 2. Leaf-drop streak of *N. rustica* caused by potato virus Y, strain Y4.

PLATE II

FIG. 3. *N. sanderae* with a necrotic disease caused by potato virus Y, strain Y3.

plants against them. However, in the work being described, some protection, which was eventually overcome, was apparent in certain strain combinations with V. Nec.

About seven to eight days after infection with V. Nec., the characteristic initial vein-clearing symptoms of virus Y developed. These were followed within two or three days by a necrosis of the midribs of the inoculated leaves. Within another two or three days, further symptoms had begun to develop in the oldest uninoculated leaf. Crinkling, distortion, and etching began at the leaf tip and progressed within about four days to the base of the leaf. After this distortion, crinkling, and etching moved from the leaf tip down about one third of the distance towards the base of this first uninoculated leaf, the second such leaf became etched and crinkled in a similar way. These symptoms developed on each leaf of the plant in the order of their age from the oldest to the youngest, but at the same time lessening in severity. When very young tobacco plants became infected they were completely destroyed within three weeks, whereas when older plants were used, there was a partial recovery as the lower severely necrotic leaves dropped off and the young or developing leaves were less and less severely affected.

Bawden and Kassanis (2) reported that their Y1 strain and the C variant of Y did not protect tobacco plants against the "tobacco veinal necrosis virus". Similarly strains Y1, Y3, Y5, Y7 of this paper and the other strains of virus Y not reported here, did not protect White Burley tobacco plants against infection by V. Nec. However, strains Y2, Y4, and Y6 did enable the tobacco plants to resist infection from V. Nec. in some degree. When V. Nec. was inoculated into plants already showing symptoms of infection with strains Y1, Y3, Y5, and Y7, the symptoms of the V. Nec. infection developed as rapidly and as severely as they did on healthy plants, and completely obliterated the original symptoms. This obliteration did not occur in plants that had been originally inoculated with the strains Y2, Y4, or Y6. The first symptoms of V. Nec. appeared about 18 days after this strain had been inoculated into the Y4- and Y6-infected plants, and about a month after it had been inoculated into the Y2-infected plants. There was no necrosis of the midrib, and the symptoms appeared in a mild form at the leaf tips as a chlorotic mottle with crinkle and some etching of the veins. The leaves were much less distorted and crinkled than in the other Y strain and V. Nec. combinations.

Discussion

Isolates of potato virus Y can only be recognized as mutants when they have ceased to cause the symptoms peculiar to this virus in certain homozygous hosts. During the course of this work the different strains have remained constant by this criterion, and there has been no evidence that mutations or changes frequently arise from this virus. However, the fact that several distinct strains were picked up in the field without difficulty indicates that changes may commonly occur.

The obvious differences between strains, as adjudged by the varying symptoms that they cause in a common host, become greater as the range of indicator plants widens. The difference between Y2 and Y3 could not have been detected without the use of *P. floridana*, or the mutual interference tests with V. Nec. on tobacco. When they separately infected any one of the large number of potato varieties used, they apparently caused the same disease within a variety, and each caused a lethal necrosis in a variety commonly considered to be tolerant to virus Y. Similarly, strains Y1 and Y4 also caused the same apparent diseases in the same varieties, including the lethal necrosis of a variety that showed only the symptoms of a mild disease when infected with Y2 and Y3. But in *P. floridana* the marked severity of Y1 is clearly differentiated from the comparatively mild effect produced by Y4. The consistency with which Y4 caused leaf-drop streak symptoms in *N. rustica* was another, but perhaps less reliable, indicator that strains Y1 and Y4 were different entities.

The differences between some of these strains and the remainder as shown by the necrotic reactions in some hosts, seem to be remarkably wide; but perhaps the differences are due to an assumption that vein-clearing followed by vein-banding is the symptom of virus Y in all *Nicotiana* species. However, this assumption has been shown to be false by the reactions of *N. sanderae* to three strains, the reaction of *N. rustica* to several strains, and the necrotic disease caused by V. Nec. on tobacco. The acceptance of V. Nec. as a strain of virus Y is based largely on the serological relationship of V. Nec. to the common field strains of virus Y (2). The fact that it does not seem to protect plants against other strains of virus Y has created some doubt (1) as to whether it is a strain of this virus. However, in the work here described, strains Y4 and Y6, which were found separately in different commercial potato varieties growing in widely separated fields, consistently demonstrated a definite, but restrained form of mutual antagonism towards V. Nec. in tobacco. This Y4 and Y6 relationship with V. Nec., together with the even greater interference to this strain from Y2, also in tobacco, indicates that these strains may have a somewhat intermediate relationship between V. Nec. and the other virus Y strains. According to Salaman (7), virus Y, as we know it, is not found in South American potato varieties. But V. Nec. is known to exist as a comparatively harmless virus of potatoes in the areas of that continent where tobacco is not grown as a common commercial crop. Perhaps it is a parent strain, and if a wider host range had been used a closer affinity to some of the common field strains of virus Y might have been observed.

As the trend in breeding for resistance in potato varieties to potato virus Y veers in a planned direction, the common strains that are truly parasitic when they infect most of the currently common varieties, may then cause a lethal necrosis in the new varieties, and so becomes self-eliminating. But there is still the possibility that new varieties may tend to encourage the easy transmission and multiplication of certain strains that at present are not common in the field.

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